FILE 'HOME' ENTERED AT 13:17:52 ON 20 FEB 2001

=> file medline biosis biotechno

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.15	0.15

FILE 'MEDLINE' ENTERED AT 13:18:30 ON 20 FEB 2001

FILE 'BIOSIS' ENTERED AT 13:18:30 ON 20 FEB 2001

FILE 'BIOTECHNO' ENTERED AT 13:18:30 ON 20 FEB 2001

=> s cr1

L1 3493 CR1

=> s cr1 and complement

L2 2441 CR1 AND COMPLEMENT

=> s l2 and fatty

L3 3 L2 AND FATTY

=> dup rem

ENTER L# LIST OR (END):13

PROCESSING COMPLETED FOR L3
L4 2 DUP REM L3 (1 DUPLICATE REMOVED)

=> d ibib abs 1-2

L4 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 94110288 MEDLINE
DOCUMENT NUMBER: 94110288
TITLE: Lipid and membrane protein transfer from human neutrophils to schistosomes is mediated by ligand binding.
AUTHOR: Rogers R A; Jack R M; Furlong S T
CORPORATE SOURCE: Department of Environmental Health, Harvard School of Public Health, Boston, MA..
CONTRACT NUMBER: AI24570 (NIAID)
NHLBI1F32 (NIAID)
AI26292
SOURCE: JOURNAL OF CELL SCIENCE, (1993 Oct) 106 (Pt 2) 485-91.
Journal code: HNK. ISSN: 0021-9533.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
AB Attachment of human neutrophils to schistosomula of Schistosoma mansoni involves leukocyte receptors recognizing carbohydrate, **complement** and/or IgG ligands on the parasite surface. Here, we examined the transfer of a fluorescent **fatty** acid analog (BOFA) from human neutrophils to schistosomula coated with concanavalin A (Con A), immune serum or

nonimmune serum under co-culture conditions by fluorescence confocal laser scanning microscopy (CLSM). Coating schistosomes with Con A or immune serum and co-culturing them for 24 hours with BOFA-labeled neutrophils resulted in a specific lipid transfer to the surface tegument of the schistosomes. Tegumental labeling was absent when nonimmune serum was used. No significant difference ($P < 0.001$) was found in the number of neutrophils bound to the worm surface between Con A-coated schistosomes (4.1 +/- 0.345 cells/worm) and worms incubated in immune serum (4.261 +/- 0.362). The number of neutrophils bound to the schistosomula (2.7 +/- 0.223) was significantly reduced in the presence of nonimmune serum ($P < 0.0001$). The viability of the schistosomula was 98% in nonimmune treated co-cultures, and 91% in cocultures treated with immune serum. HPLC analysis of labeled neutrophils demonstrated that BOFA was incorporated into both phospholipids and neutral lipids, which were almost exclusively triglycerides and, after 18 hours of culture, all of the **fatty** acid analog was incorporated into complex lipids. Double-label experiments in which schistosomula bearing Con A were first incubated with BOFA-labeled neutrophils and subsequently immunolabeled revealed that the neutrophil membrane proteins, MHC class I, **CR1** and CR3 were co-transferred with neutrophil lipids to the parasite tegument.(ABSTRACT TRUNCATED AT 250 WORDS)

L4 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:455632 BIOSIS
DOCUMENT NUMBER: BA92:100412
TITLE: LEUKOTRIENE B-5 IMPORTANCE AND PERSPECTIVES IN DERMATOLOGY.
AUTHOR(S): WOZEL G; BARTH J
CORPORATE SOURCE: KLINIK HAUTKRANKHEITEN MEDIZINISCHEN AKADEMIE CARL GUSTAV CARUS, FETSCHERSTR. 74, O-8019 DRESDEN, BUNDESREPUBLIK DEUTSCHLAND.
SOURCE: DERMATOL MONATSSCHR, (1991) 177 (3), 157-166.
CODEN: DMONBP. ISSN: 0011-9083.
FILE SEGMENT: BA; OLD
LANGUAGE: German
AB Leukotriene B5 (LTB5) is a twofold hydroxylated **fatty** acid with twenty carbons and five double bonds. In human beings LTB5 is biosynthesized by 5-lipoxygenase from eicosapentaenoic acid (EPA). EPA is found in large amounts in diet rich in cold-water fish oil and in special dietary supplementation. LTB5 binds with different affinity the two subclasses of LTB4-receptors. With respect to the biological activity LTB5 is considerably less active than LTB4, for example, considering the leukocyte functions like aggregation, chemotaxis, and expression of **complement** receptors (**CR1**, CR3). As arachidonic acid (AA) and EPA is competitively metabolized by the same enzymes of AA-cascade LTB5 effects inhibition of LTB4 production and exhibits, therefore, antiinflammatory activity. Additionally, EPA/LTB5 suppresses the synthesis of cytokines like interleukin-1 and tumor necrosis factor. Recent clinical experiences EPA/LTB5 reveal therapeutic potential in inflammatory dermatological (e.g. psoriasis) and other disorders and justify further experimental investigations.

=> d his

(FILE 'HOME' ENTERED AT 13:17:52 ON 20 FEB 2001)

FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 13:18:30 ON 20 FEB 2001
L1 3493 S CR1
L2 2441 S CR1 AND COMPLEMENT
L3 3 S L2 AND FATTY
L4 2 DUP REM L3 (1 DUPLICATE REMOVED)

=> s l1 and fatty

L5 10 L1 AND [redacted]TY

=> s l5 not l3

L6 7 L5 NOT L3

=> dup rem l6

PROCESSING COMPLETED FOR L6
L7 7 DUP REM L6 (0 DUPLICATES REMOVED)

=> d ibib abs

L7 ANSWER 1 OF 7 MEDLINE
ACCESSION NUMBER: 95383320 MEDLINE
DOCUMENT NUMBER: 95383320
TITLE: Fe(III)-lipoxygenase converts its suicide-type inhibitor octadeca-9,12-diynoic acid into 11-oxooctadeca-9,12-diynoic acid.
AUTHOR: Nieuwenhuizen W F; Schilstra M J; van der Kerk-Van Hoof A; Brandsma L; Veldink G A; Vliegenthart J F
CORPORATE SOURCE: Bijvoet Center for Biomolecular Research, Department of Bio-Organic Chemistry, Utrecht University, The Netherlands.
SOURCE: BIOCHEMISTRY, (1995 Aug 22) 34 (33) 10538-45.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
AB Triple bond analogues of polyunsaturated **fatty** acids irreversibly inactivate lipoxygenases. During the inactivation the inhibitors are converted enzymatically [Kuhn, H., et al. (1984) Eur. J. Biochem. 139, 577-583]. Since the converted inhibitor molecules may hold important information about the inactivation mechanism, we have determined the structure of the product that is formed during the irreversible inactivation of soybean lipoxygenase-1 by octadeca-9,12-diynoic acid (ODYA), the triple bond analogue of linoleic acid. This product is formed only in the presence of Fe(III)-lipoxygenase-1 and O2. It was purified by C18 solid phase extraction and reversed phase HPLC and was identified with UV, IR, and NMR spectroscopic and mass spectrometric techniques as the novel lipoxygenase product, 11-oxooctadeca-9,12-diynoic acid (11-oxo-ODYA). It is estimated that each lipoxygenase molecule produces 8-10 11-oxo-ODYA molecules before it is inactivated. Furthermore, we have shown that in a secondary reaction 3-4 molecules of 11-oxo-ODYA are covalently attached per lipoxygenase molecule, most likely, to solvent-exposed amino groups. This leads to the formation of a N-penten-4-yn-3-one chromophore, RC(NHX)=CHC(O)C=**CR1**, in which X stands for the protein and R or R1 for CH3(CH2)4- or -(CH2)7COOH, respectively. Fe(II)- and Fe(III)-lipoxygenase remain active upon reaction with purified 11-oxo-ODYA. It is concluded that (a) several enzymatic turnovers are required for the complete inactivation of lipoxygenase by ODYA and (b) covalent attachment of 11-oxo-ODYA occurs outside the active site and is not the cause of the inactivation.

=> d ibib abs 2-7

L7 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:265726 BIOSIS
DOCUMENT NUMBER: PREV199497278726
TITLE: In vitro culture of bovine IVM-IVF embryos: Cooperative

interaction among embryos and the role of growth factors.
AUTHOR(S): [redacted], C. L. (1); Stice, S. L.; [redacted]ki, A. M.; Golueke, P.
CORPORATE SOURCE: (1) ABS Specialty Genetics, American Breeders Serv., 6908 River Rd., DeForest, WI 53532-0459 USA
SOURCE: Theriogenology, (1994) Vol. 41, No. 6, pp. 1323-1331. ISSN: 0093-691X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The objective of this study was to determine whether there is a cooperative interaction among bovine embryos during in vitro culture. Furthermore, culture medium was supplemented with the growth factors, epidermal growth factor (EGF) and transforming growth factor-beta-1 (TGF-beta-1), to determine if these factors had a stimulatory effect on bovine embryo development similar to that seen in mouse development. In vitro matured - in vitro fertilized bovine embryos (2- to 8-cell) were cultured singly and in groups of five in 25 mu-l of medium (**CR1** + amino acids + **fatty** acid-free bovine serum albumin) with or without EGF and TGF-beta-1. Bovine embryos cultured in groups had a significantly higher rate of development to the blastocyst stage than embryos cultured singly. Neither EGF (10 ng/ml) nor TGF-beta-1 (2 ng/ml) affected blastocyst development, hatching or the cell number of the embryos cultured in groups. Epidermal growth factor stimulated hatching of embryos cultured singly from the 8-cell stage, but did not significantly affect blastocyst development.

L7 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:366907 BIOSIS
DOCUMENT NUMBER: PREV199497379907
TITLE: Changes in the liver, kidney and heart **fatty** acid composition following administration of ibuprofen to mice.
AUTHOR(S): Tvrzicka, E. (1); Cvrckova, E.; Maca, B.; Jiraskova, M.
CORPORATE SOURCE: (1) First Fac. Med., 4th Dep. Med., Charles Univ., U. nemocnice 2, 128 08 Prague Czech Republic
SOURCE: Journal of Chromatography B Biomedical Applications, (1994) Vol. 656, No. 1, pp. 51-57.
DOCUMENT TYPE: Article
LANGUAGE: English
AB **Cr1**:NMRI-BR male mice received 0.6 mg/day of ibuprofen (animal model for human dose 1200 mg/day) in the diet for a period of 6 weeks. This treatment resulted in increased body mass, liver mass, and total lipid content in the liver tissue. Changes in the **fatty** acid composition in the individual lipid classes were most important in kidney tissue; levels of polyunsaturated **fatty** acids were increased in phospholipids and decreased in neutral lipids. These changes were compensated for by opposite changes in the levels of saturated and monoenoic acids. Similar changes were also observed in liver and heart lipids. An increased level of an unusual component was observed in heart tissue, which was identified as isopropyl myristate by GC-MS and verified by comparing the mass spectra and retention times with those of synthetic standards.

L7 ANSWER 4 OF 7 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:140135 BIOSIS
DOCUMENT NUMBER: PREV199395072935
TITLE: Hexanal production and TBA number are reduced in soybean (Glycine max (L.) Merr.) seeds lacking lipoxygenase isozymes 2 and 3.
AUTHOR(S): Moreira, Maurilio A. (1); Tavares, Sebastiao R.; Ramos, Virginia; De Barros, Everaldo G.
CORPORATE SOURCE: (1) Bioagro, Univ. Federal de Vicosa, 36570 Vicosa, MG Brazil
SOURCE: Journal of Agricultural and Food Chemistry, (1993) Vol. 41, No. 1, pp. 103-106. ISSN: 0021-8561.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Lipoxygenase (LOX) isozyme (linoleate:oxygen oxidoreductase, EC 1.13.11.12) catalyze the hydroperoxidation of unsaturated **fatty** acids with a cis,cis-1,4-pentadiene moiety. This reaction is the first step leading to the production of several compounds which appear to be directly related with the off-flavor characteristic of soybean protein products. In our breeding program toward cultivars yielding better tasting protein products, we determined that hexanal production and 2-thiobarbituric acid (TBA) number for the variety Cristalina (CR) and for lines derived from Cristalina lacking LOX 1 (**CR1**), LOX 2 (CR2), LOX 3 (CR3), LOX 1,3 (**CR1**,3), and LOX 2,3 (CR2,3). Removal of LOX 1 did not affect hexanal production or TBA number. However, removal of LOX 3 caused a significant decrease on both parameters. LOX 2 proved to be the most important isozyme for hexanal production. The double-recessive line lacking LOX 2 and LOX 3 yielded the lowest TBA numbers and hexanal levels. This was true for seeds with moisture contents ranging from 10.9 to 21.3%.

L7 ANSWER 5 OF 7 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:50700 BIOSIS
DOCUMENT NUMBER: PREV199395027002
TITLE: Effects of linoleic free acid, methyl linoleate and trilinolein on the production of carbonyl compounds in soybean seed homogenates.
AUTHOR(S): Pereira, Maristela (1); Moreira, Maurilio A. (1); Rezende, Sebastiao T. (1); Sediyama, Carlos S.
CORPORATE SOURCE: (1) Dep. Quimica, Univ. Federal de Vicosa, 36570-000 Vicosa, Minas Gerais
SOURCE: Arquivos de Biologia e Tecnologia (Curitiba), (1992) Vol. 35, No. 2, pp. 403-419.
ISSN: 0365-0979.
DOCUMENT TYPE: Article
LANGUAGE: Portuguese
SUMMARY LANGUAGE: English

AB In the seeds of soybean, there are namely three lipoxygenase isozymes, LOX 1, LOX 2 and LOX 3. They catalyze the hydroperoxidation of polyunsaturated **fatty** acids containing cis, cis-1, 4-pentadiene moieties, producing 9 or 13-cis, trans hydroperoxides which are enzymatically degraded into acids, aldehdyes, ketones, alcohols and hydrocarbons of short carbon chains. These compounds respond for the beany flavor normally associated with soybean products. This work was performed to evaluate the efficiency of the substrates linoleic free acid methyl linoleate and trilinolein, added to soybean extracts, on the production of carbonyl compounds as determined by the TBA number. The experiments were conducted with crude soybean extracts obtained from seeds of Cristalina -L1 (**CR1**), Cristalina -L2 (CR2), Cristalina -L3 (CR3) and Cristalina -L1 -L3 (**CR1**,3) and Cristalina -L2 -L3 (CR2,3) in different pH values. The results showed that more carbonyl compounds could be detected when methyl linoleate and trilinolein were added to extracts containing LOX 2 and/or LOX 3 and not LOX-1, at neutral pH. On the other hand, extracts containing only LOX 1 were more effective in producing carbonyl compound when linoleic free acid was added, in alkaline medium. It became also evident that the genetic elimination of LOX 2 and LOX 3 makes LOX 1 to be more effective on this regard as compared to the normal variety. It could also be demonstrated that, in general, LOX 2 is more effective on the production of carbonyl compounds, in neutral pH, no matter the substrate added to the reaction mixture.

L7 ANSWER 6 OF 7 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:56066 BIOSIS
DOCUMENT NUMBER: PREV199395032368

TITLE: Effects of linoleic free acid, methyl linoleate and trilinolein, on the hexanal production in soybean homogenates.
AUTHOR(S): Pereira, Maristela (1); Moreira, Maurilio A. (1); Rezende, Sebastiao T. (1); Sediyama, Carlos S.
CORPORATE SOURCE: (1) Dep. Quimica, Univ. Federal de Vicosa, 36570 Vicosa, Minas Gerais
SOURCE: Arquivos de Biologia e Tecnologia (Curitiba), (1992) Vol. 35, No. 2, pp. 389-402.
ISSN: 0365-0979.
DOCUMENT TYPE: Article
LANGUAGE: Portuguese
SUMMARY LANGUAGE: English

AB It is well known that the action of soybean seed lipoxygenases upon polyunsaturated **fatty** acids brings to the formation of hexanal, one of the major carbonyl compounds found in the reaction mixture. In this work, the contribution of each of the soybean lipoxygenases was evaluated, on the hexanal production, by adding linoleic free acid, methyl linoleate, and trilinolein, to crude extracts of Cristalina -L1 (**CR1**), Cristalina -L2 (CR2), Cristalina -L3 (CR3), Cristalina -L1 -L3 (**CR1**,3) and, Cristalina -L2 -L3 (CR2,3) at different pH values. Hexanal was measured, in the reaction mixture, by head space gas chromatograph. LOX 2 showed more activity in pH values below 7,0, upon addition of linoleic free acid, while LOX 1 demonstrated more activity at basic medium. Upon genetic elimination of LX 2 and LOX 3 (CR2,3), LOX 1 became more effective on the evolution of hexanal under addition of linoleic free acid. With methyl linoleate, only trace amounts of hexanal could be detected in the reaction mixtures, this might be due to weak solubility of the substrate in all pHs used. Experiments were conducted to compare the behaviour of **CR1** and CR2,3 with triglycerides, on pH 7,0 and 9,0. LOX 2 and LOC 3 were clearly more effective than LOX 1, on hexanal production, after the addition of trilinolein.

L7 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:26039 BIOSIS
DOCUMENT NUMBER: BA91:15390
TITLE: SOY MILK FLAVOR LIPOXYGENASE AND HEXANAL.
AUTHOR(S): GOMES J C; DA SILVA M V; COELHO D T; CHAVES J B P; REZENDE S T
CORPORATE SOURCE: DEP. TECNOLOGIA ALIMENTOS, UNIV. FEDERAL VICOSA, 36570 VICOSA, MG.
SOURCE: ARQ BIOL TECNOL (CURITIBA), (1990) 33 (2), 353-368.
CODEN: ABTTAP. ISSN: 0365-0979.
FILE SEGMENT: BA; OLD
LANGUAGE: Portuguese

AB Lipoxygenase isozymes have been considered as major contributors to the lipid fraction instability during storage and processing of soybean. The lipoxygenases designated by L1, L2 and L3 catalyse the hydroperoxidation in groups 1-4-Cis-Cis pentadiene in linoleic and linolenic **fatty** acids. These chemical changes yield secondary products which are believed to be responsible for the flavor of soy products. It was intended in this research to evaluate the effects of the genetic elimination of isozymes L1 and L3 activities on soybean hydrosoluble extracts (soy milk) fresh and powder rehydrated sensory qualities. Soy milk was obtained from soybeans of "Cristalina" (CR) variety and from soybeans of the third backcross genotypes designated as **CR1** and CR3, which are deprived from isozymes L1 and L3 activities. Hexanal and malonaldehyde levels, and sensory evaluation were used to access off-flavor development in soybean hydrosoluble extracts. Hexanal and malonaldehyde levels in soybean hydrosoluble fresh and dry rehydrated extracts from the **CR1** genotype were greater than those found in the hydrosoluble extracts from Cristalina variety; while lower values were observed for soybean hydrosoluble extracts obtained from CR3 genotypes. Sensory evaluation rank

data, from a nine member trained panel, led to the conclusion that soybean
fresh hydrosoluble extract from CR3 genotype was rated better, and the dried rehydrated hydrosoluble extract from **CR1** genotype was rated worst ($P < 0,05$). These sensory evaluation data are in agreement with the chemical analysis; the hydrosoluble extract from **CR1** genotype presented higher hexanal and malonaldehyde levels than that from CR3 genotype. Based on data from this experiment, it can also be concluded
that hexanal and malonaldehyde levels in soybean hydrosoluble extract are appropriate in the evaluation of its characteristic soybean flavor.

=> s l2 and conjug?

L8 36 L2 AND CONJUG?

=>

=> dup rem l8

PROCESSING COMPLETED FOR L8
L9 19 DUP REM L8 (17 DUPLICATES REMOVED)

=> d ibib abs 1-19

L9 ANSWER 1 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:412641 BIOSIS
DOCUMENT NUMBER: PREV200000412641
TITLE: Quantitative assay of **complement** receptor 1 expressed on erythrocytes and the significance in clinic.
AUTHOR(S): Wang Haibin (1); Zhang Jingping; Wang Hui
CORPORATE SOURCE: (1) Department of Immunology, Second Military University, Changhai Hospital, Shanghai, 200433 China
SOURCE: Zhonghua Weishengwuxue He Mianyixue Zazhi, (July, 2000) Vol. 20, No. 4, pp. 381-384. print.
ISSN: 0254-5101.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
SUMMARY LANGUAGE: Chinese; English
AB Objective: To investigate the role of **CR1** on erythrocytes(ECR1) in immune-related diseases, we have developed a highly sensitive and reproductive technique to the examine number of the **complement** receptor 1 on erythrocytes (ECR1). Methods: Glutaraldehyde-fixed red blood
cells (RBC) were analysed in V well microtiter plates. To the cell buttons
in each well, mouse monoclonal anti-**CR1** was added and the microtiter was incubated at 37degreeC for 50 min, followed by three washes
in S/BSA. The anti-mouse IgG, **conjugated** to alkaline phosphatase was then added, and was incubated in the same condition. Substrate was added and incubated at 37degreeC for 90 min. The supernatant was transferred to a clean U microtiter plate to facilitate reading in a plate
reader at 405nm (A). Analysis the relationship between the A and the diseases. Results: The method can nicely examine the quantity of **CR1** on RBC and has a good sensitivity and reproducibility in methodology. The results showed that the ECR1 expression on RBC from patients with hepatocellular cancer (HCC), large intestine cancer (LIC), rheumatic cardiopathy (RC), ovary cancer(OC) and systemic lupus erythematosus (SLE) was significantly lower than that of healthy individuals($P < 0.01$). In particular, ECR1 on RBC in patients with SLE is significantly lower than that of patients with other diseases ($P < 0.01$). Furthermore, the **CR1** expressed on RBC from the patients of SLE is obviously related to the development of the disease and the efficiency of treatment. Conclusions: The technique has enabled us to investigate the

role of ECR1 in immune-related diseases. The results suggest that the expression of ECR1 may be involved in pathogeny, development and prognosis of the diseases. It is very important to analyse the quantity of ECR1 in diagnosis and the evaluation of treatment of diseases.

L9 ANSWER 2 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:389132 BIOSIS
DOCUMENT NUMBER: PREV200000389132
TITLE: Immunomodulatory functions of murine **CR1**/2.
AUTHOR(S): Prechl, Jozsef; Erdei, Anna (1)
CORPORATE SOURCE: (1) Department of Immunology, Eotvos Lorand University, God, Javorra Sandor u.14, Budapest Hungary
SOURCE: Immunopharmacology, (August, 2000) Vol. 49, No. 1-2, pp. 117-124. print.
ISSN: 0162-3109.
DOCUMENT TYPE: General Review
LANGUAGE: English
SUMMARY LANGUAGE: English
AB C3-fragments generated upon **complement** activation play an important role in the formation and regulation of immune responses. Receptors interacting with various activation fragments of this versatile **complement** component are expressed on a wide variety of cell types, such as lymphocytes, macrophages, dendritic cells, follicular dendritic cells, granulocytes, erythrocytes, consequently C3-products may influence several biological functions at different sites of the body, where **complement** activation takes place. In the last decade, genes, protein structure and functions played by murine **complement** receptors **CR1** and CR2 (mCR1/2) have been deciphered. In this review, we wish to relate these properties, and fit it into the context of events following in vivo **complement** activation. We separately address the roles played by murine mCR1/2 as BCR coreceptor and as BCR independent structure, and propose a mchanism for the utilization of antigen-C3d **conjugates** bound on B cells. Finally, we raise some of the questions that remain to be elucidated in order to get a more precise picture of the functions of mCR1/2.

L9 ANSWER 3 OF 19 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999192721 MEDLINE
DOCUMENT NUMBER: 99192721
TITLE: Modulation of the humoral immune response by antibody-mediated antigen targeting to **complement** receptors and Fc receptors.
AUTHOR: Baiu D C; Prechl J; Tchorbanov A; Molina H D; Erdei A; Sulica A; Capel P J; Hazenbos W L
CORPORATE SOURCE: Department of Immunology, University Hospital Utrecht, The Netherlands.
SOURCE: JOURNAL OF IMMUNOLOGY, (1999 Mar 15) 162 (6) 3125-30. Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199906
ENTRY WEEK: 19990603
AB During an ongoing immune response, immune complexes, composed of Ag, **complement** factors, and Igs, are formed that can interact with **complement** receptors (CRs) and IgG Fc receptors (Fc gamma R). The role of **CR1**/2 and Fc gamma R in the regulation of the immune response was investigated using OVA that was chemically **conjugated** to whole IgG of the rat anti-mouse **CR1**/2 mAb 7G6. FACS analysis using the murine B cell lymphoma IIA1.6 confirmed that the 7G6-OVA **conjugate** recognized **CR1**/2. Incubating IIA1.6 cells with 7G6-OVA triggered tyrosine phosphorylation and Ag presentation to OVA-specific T cells in vitro. Immunizing mice with 7G6-OVA at a minimal dose of 1 microgram i.p. per mouse markedly enhanced the anti-OVA Ig response, which was primarily of the IgG1 isotype subclass. The 7G6-OVA

did not enhance the anti-OVA response in **CR1**/2-deficient mice. OVA coupled to an [redacted] type control Ab induced a considerably lower anti-OVA response compared with that induced by OVA alone, suggesting inhibition by interaction between the Fc part of the Ab and the inhibitory Fc gamma RIIb on B cells. This findings was supported by the observation that IIA1.6 cells which were incubated with 7G6-OVA lost the ability to present Ag upon transfection with Fc gamma RIIb. In sum, 7G6-**conjugated** OVA, resembling a natural immune complex, induces an enhanced anti-OVA immune response that involves at least **CR1**/2-mediated stimulation and that may be partially suppressed by Fc gamma RIIb.

L9 ANSWER 4 OF 19 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999333833 MEDLINE
DOCUMENT NUMBER: 99333833
TITLE: Low responsiveness to immunization with immunoglobulin E/antigen and immunoglobulin G/antigen complexes in H-2Ab mice.
AUTHOR: Gustavsson S; Chomez S; Heyman B
CORPORATE SOURCE: Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden.
SOURCE: SCANDINAVIAN JOURNAL OF IMMUNOLOGY, (1999 Jul) 50 (1) 45-51.
Journal code: UCW. ISSN: 0300-9475.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199910
ENTRY WEEK: 19991003
AB Immunoglobulin (Ig)E and IgG antibodies specific for 2,4, 6-trinitrophenyl (TNP) are able to enhance the carrier-specific antibody response to TNP-**conjugated** soluble proteins such as bovine serum albumin (BSA). We have recently reported that mice carrying the MHC class II Ab molecule are low responders to immunization with IgE/antigen complexes and now show that H-2Ab mice are also low responders to IgG/antigen complexes. In addition, we found that spleen cells from naive low- and high-responder mice captured IgE/antigen complexes exclusively on B cells, and that the binding was completely inhibited by monoclonal antibodies (MoAbs) against the low-affinity receptor for IgE (FcepsilonRII or CD23). The IgG/antigen complexes were targeted both to B cells and macrophages. The binding of IgG/antigen to B cells primarily seemed to be dependent on the low-affinity receptor for IgG (FcgammaRII or CD32), although some influence of **complement** receptor 2 (CR2 or CD21) was seen. Capture of IgG/antigen complexes on macrophages was partially blocked by MoAbs against FcgammaRII/III. There was no difference in expression of FcepsilonRII, FcgammaRII/III, **CR1**, CR2, and CR3 between low- and high-responder strains, thus excluding low levels of these FcRs and CRs as a reason for low responsiveness in H-2Ab mice.

L9 ANSWER 5 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:363094 BIOSIS
DOCUMENT NUMBER: PREV199800363094
TITLE: Expression of **complement**-related membrane proteins on lymphocytes and alveolar macrophages in bronchoalveolar lavage fluid.
AUTHOR(S): Nakahara, Risa (1); Takemura, Shuhei; Onodera, Hideki; Kasamatsu, Yoshihiro; Seto, Nobuyuki; Ichio, Naoko; Doi, Takashi; Nakanishi, Sadanobu; Okamoto, Masayuki; Yanagida, Kunio; Ueda, Masahiro; Deguchi, Masako; Kondo, Motoharu
CORPORATE SOURCE: (1) First Dep. Intern. Med., Kyoto Pref. Univ. Med., 465 Kajiimachi, Hirokojiagaru, Kawaramachi-dori, Kamigyo-ku, Kyoto 602 Japan
SOURCE: Allergology International, (June, 1998) Vol. 47, No. 2, pp.

109[illegible]6.
ISS[illegible]1323-8930.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Interstitial lung disease (ILD) can be separated into two groups: (i) that in which the causes are known; and (ii) that in which the etiologies have not been determined. Meanwhile, **complement** is essential for the inflammatory response and **complement** systems have been recognized as factors involved in organizing ILD. In the present study we investigated whether there were any differences in **complement**-related membrane proteins on lymphocytes and alveolar macrophages in bronchoalveolar lavage fluids (BALF) between different ILD. Bronchoalveolar lavage fluid samples were obtained from 12 patients and eight healthy individuals. Bronchoalveolar lavage fluid cells were incubated with monoclonal antibodies (mAbs) against C3b/C4b receptor (**CR1**), C3bi receptor (CR3), membrane cofactor protein (MCP) and decay accelerating factor (DAF) and were then labeled with fluorescein isothiocyanate-**conjugated** anti-mouse IgG. Cells were analyzed using a flow cytometer.The results demonstrate for the first time that CR3 and DAF are elevated on alveolar macrophages, and that MCP and DAF are increased on BALF lymphocytes in sarcoidosis. The possible roles of **complement**-related membrane proteins in the pathogenesis of immune processes that are ongoing in sarcoidosis are still obscure, but our results may provide some useful information on the mechanisms underlying sarcoidosis.

L9 ANSWER 6 OF 19 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97461942 MEDLINE
DOCUMENT NUMBER: 97461942
TITLE: The monoclonal antibody-specific immobilization of erythrocyte antigens assay (MAIEA) in the investigation of human red-cell antigens and their associated membrane proteins.
AUTHOR: Petty A C; Green C A; Daniels G L
CORPORATE SOURCE: Bristol Institute for Transfusion Sciences, UK.
SOURCE: TRANSFUSION MEDICINE, (1997 Sep) 7 (3) 179-88. Ref: 49
Journal code: BU7. ISSN: 0958-7578.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY WEEK: 19980104
AB The monoclonal antibody-specific immobilization of erythrocyte antigens (MAIEA) technique is an immunoassay devised primarily for locating blood group antigens on specific red-cell membrane proteins. The assay involves the incubation of intact red cells with two antibodies, one human alloantibody, the other a nonhuman antibody, usually a rodent monoclonal antibody, but polyclonal antibodies of rabbit origin have been utilized. For a positive result, both antibodies must bind to the same membrane protein. The red cells are lysed, the membrane solubilized and the trimolecular complex of two antibodies and membrane protein is captured in a well coated with goat antirodent (or rabbit) immunoglobulin. The immobilized complex is then detected by the use of peroxidase-**conjugated** goat antihuman (or rodent) immunoglobulin. Negative results, due to mutual blocking between the human and animal antibodies when their epitopes are close together on the same molecule, have permitted a degree of localization of epitopes on some proteins. This has been most effective in the mapping of Cromer blood group system antigens on the **complement** control protein domains of decay-accelerating factor (DAF, CD55), but has also proved informative in the clustering of antigens on the Lutheran and Kell glycoproteins. MAIEA is an effective tool for the identification of antibodies to Knops-system antigens on **complement** receptor 1 (**CR1**, CD35) in immunohaematology

reference laboratories. These antibodies are clinically unimportant, but must be identified before they can be ignored for transfusion purposes.

L9 ANSWER 7 OF 19 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97064127 MEDLINE
DOCUMENT NUMBER: 97064127
TITLE: **Complement**-dependent binding of C-reactive protein complexes to human erythrocyte **CR1**.
AUTHOR: Mold C; Gurule C; Otero D; Du Clos T W
CORPORATE SOURCE: Department of Microbiology, University of New Mexico, Albuquerque 87131, USA.
CONTRACT NUMBER: SO6 GM-08139 (NIGMS)
RO1 AR-42538 (NIAMS)
R29 AI-28358 (NIAID)
SOURCE: CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY, (1996 Nov) 81 (2) 153-60.
Journal code: DEA. ISSN: 0090-1229.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199703
ENTRY WEEK: 19970301
AB C-reactive protein (CRP) is an acute phase serum protein that binds to phosphocholine (PC) on phospholipids and polysaccharides and to protein components of chromatin and small nuclear ribonucleoproteins. Complexes between CRP and ligands activate **complement** and bind to receptors on phagocytic cells. Although **complement** is required for CRP-mediated clearance or phagocytosis of ligand-coated erythrocytes, the participation of **complement** and **complement** receptors in clearance of soluble CRP complexes has not been examined. We have used PC-**conjugated** BSA to prepare complexes containing either IgG antibody or CRP. We found similar **complement**-mediated binding of both types of complexes to human erythrocyte **complement** receptors (**CR1**, CD35). We also found that serum deficient in C4A or C4B supported binding of CRP and IgG complexes to erythrocytes. These findings indicate that complexes between CRP and soluble ligands may be cleared by the erythrocyte **CR1** pathway described for soluble immune complexes.

L9 ANSWER 8 OF 19 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 96389162 MEDLINE
DOCUMENT NUMBER: 96389162
TITLE: Rapid purification of human **complement** receptor type 1 (CD35, **CR1**).
AUTHOR: Seya T; Matsumoto M; Hatanaka M; Okada M; Masaki T; Iida K
CORPORATE SOURCE: Department of Immunology, Center for Adult Diseases Osaka, Japan.
SOURCE: JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, (1996 May 14) 32 (2) 69-76.
Journal code: H94. ISSN: 0165-022X.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY WEEK: 19970104
AB We established a rapid purification procedure for **complement** receptor type 1 (**CR1**, CD35). Human erythrocyte stromata were solubilized, and the extract was directly applied to a Red-Sepharose column. The eluate was diluted 2-fold, then subjected to an immunoaffinity
column, anti-**CR1** (named 31R)-**conjugated** to Sepharose. More than 50% of **CR1** was recovered with purity of > 90%. The **CR1** preparation showed sufficient cofactor activity for factor I as compared to that prepared by the conventional method. Since 31R recognizes a single epitope in **CR1** including its rare variants and soluble forms, this method will allow us to recover with high efficiency these forms of **CR1** which have been detected

especially in some sease states.

L9 ANSWER 9 OF 19 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 95386972 MEDLINE
DOCUMENT NUMBER: 95386972
TITLE: Lipopolysaccharide (LPS)-specific monoclonal antibodies regulate LPS uptake and LPS-induced tumor necrosis factor-alpha responses by human monocytes.
AUTHOR: Pollack M; Espinoza A M; Guelde G; Koles N L; Wahl L M; Ohl
C A
CORPORATE SOURCE: F. Edward Hebert School of Medicine, Dept. of Medicine, USUHS, Bethesda, MD 20814-4799, USA.
CONTRACT NUMBER: AI-22706 (NIAID)
SOURCE: JOURNAL OF INFECTIOUS DISEASES, (1995 Sep) 172 (3) 794-804.
Journal code: IH3. ISSN: 0022-1899.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199512
AB Lipopolysaccharide (LPS)-monocyte/macrophage interactions are central to the infected host's inflammatory response to gram-negative bacteria. Flow cytometry was used to analyze the regulation by LPS-specific monoclonal antibodies (MAbs) of fluorescein isothiocyanate-**conjugated** LPS uptake by human peripheral blood monocytes. The uptake of LPS was stimulated by fresh or heat-inactivated serum (NHS or delta NHS) or by LPS-binding protein and inhibited by alpha-LPS or alpha-CD14 (LPS receptor) MAbs. The inhibition of alpha-LPS uptake was offset in the presence of NHS by a simultaneous MAb-mediated increase in LPS uptake that was blocked by alpha-**complement** receptor 1. Monocyte tumor necrosis factor-alpha responses to LPS were augmented by NHS and delta NHS and inhibited by alpha-LPS MAbs. Thus, alpha-LPS MAbs down-regulate the proinflammatory uptake of LPS by human monocytes via membrane-bound CD14 while promoting **complement**-mediated opsonic uptake through membrane-associated **CR1**.

L9 ANSWER 10 OF 19 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 94365178 MEDLINE
DOCUMENT NUMBER: 94365178
TITLE: Internalization of type 1 **complement** receptors and de novo multivesicular body formation during chemoattractant-induced endocytosis in human neutrophils.
AUTHOR: Berger M; Wetzler E; August J T; Tartakoff A M
CORPORATE SOURCE: Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106..
CONTRACT NUMBER: AI22687 (NIAID)
RR05410-29 (NCRR)
DK38181 (NIDDK)
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1994 Sep) 94 (3) 1113-25.
Journal code: HS7. ISSN: 0021-9738.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199412
AB Upon activation of human neutrophils by chemoattractants, functionally important proteins are rapidly transported from intracellular granules and storage vesicles to the plasma membrane. This is accompanied by a marked increase in the rate of endocytosis and by ligand-independent internalization of type 1 **complement** receptors (**CR1**).
To define the pathway of endocytosis, we used gold-**conjugated** BSA in a pulse-chase protocol. This tracer was initially internalized into

small endocytic ve[redacted]les which rapidly traversed th[redacted]ytoplasm and coalesced to form l[redacted]ge, conspicuous multivesicular [redacted]dies. Within 5 min after addition of the chemoattractant, multivesicular bodies contained > 60% of the cell-associated BSA-gold. **CR1** colocalized with the endocytic tracer in both the early endosomes and multivesicular bodies. In unstimulated cells, there was much less uptake of BSA-gold and multivesicular bodies were rarely seen. Using the acidotropic amine, DAMP, and anti-DNP antibodies, we found that the multivesicular bodies were acidified but the early endosomes did not concentrate DAMP. Neither the early endosomes nor the multivesicular bodies initially contained the lysosomal membrane antigens hLAMP 1 or 2, but hLAMP-positive structures subsequently joined the multivesicular bodies. The rapid activation of the endocytic pathway upon stimulation of neutrophils allowed us to visualize the de novo formation and maturation of multivesicular bodies. Our observations suggest that vesicles containing ion pumps and acid hydrolases fuse with multivesicular bodies, giving them characteristics of lysosomes, and that these are the probable sites of degradation of **CR1**. The observations do not support models which would require transport of **CR1** from multivesicular bodies to defined, pre-existing lysosomes for degradation.

L9 ANSWER 11 OF 19 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 93100509 MEDLINE
DOCUMENT NUMBER: 93100509
TITLE: Expression of the DAF (CD55) and CD59 antigens during normal hematopoietic cell differentiation.
AUTHOR: Terstappen L W; Nguyen M; Lazarus H M; Medof M E
CORPORATE SOURCE: Becton Dickinson Immunocytometry Systems, San Jose, CA 95131..
SOURCE: JOURNAL OF LEUKOCYTE BIOLOGY, (1992 Dec) 52 (6) 652-60. Journal code: IWY. ISSN: 0741-5400.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199303

AB Expression of decay-accelerating factor (DAF or CD55) and of CD59 during hematopoietic cell development in normal bone marrow and on peripheral blood leukocytes were characterized by three-color immunofluorescence experiments. With this technique cell subsets were identified by forward light scatter, orthogonal light scatter, and two cell-surface antigens. For each cell lineage, specific combinations of two monoclonal antibodies labeled with different fluorochromes were used. DAF or CD59 were then quantitated on the defined cell subsets from the fluorescence signal of the respective antibody **conjugated** with a third fluorochrome. Early uncommitted hematopoietic progenitor cells (CD34+, CD38-) all expressed both proteins homogeneously. Initial commitment to the erythroid (CD71+, CD45dim), myeloid (CD33+), or B lymphocyte (CD10+) lineages was not associated with changes in DAF or CD59 levels. With erythroid development, i.e., after loss of CD45 and decrease of CD71, expression of both proteins decreased. With myeloid maturation, expression of CD59 remained constant and expression of DAF varied. During neutrophil maturation, DAF decreased initially and then reemerged on maturing neutrophils concurrently with the appearance of CD16 (Fc gamma RIII), whereas during monocyte maturation, DAF increased concurrently with up-regulation of CD14. With B cell development, expression of DAF increased concurrently with down-regulation of CD10 and up-regulation of CD20, whereas expression of CD59 diminished slightly late in B cell maturation. Analysis of peripheral blood elements showed that monocytes, neutrophils, and B lymphocytes expressed both proteins homogeneously, but that in contrast to other cell subsets, which all expressed CD59, only a subset of (CD3+) T lymphocytes and (CD16+) Natural killer cells expressed DAF. The absence of DAF was not related to CD4 or CD8 expression or to the

presence of activation markers (CD25+, CD38+), memory cell markers (CD58+,
CD45RO+), or virgin T cell markers (CD45RA+), but was correlated with expression of CD11b (CR3) and CD11c (gp150/95). Although CD21+ (CR2) and CD35+ (**CR1**) cells all expressed DAF, CD11a (LFA-1) levels correlated inversely with those of DAF. Although the presence of CD55 and CD59 on early progenitor cells and throughout hematopoietic cell development is consistent with the requirements for both proteins in protection of host cells from **complement**-mediated injury, the physiological relevance of the unique patterns of variation for each cell lineage is unclear. Nevertheless, the availability of a detailed DAF and CD59 expression map in normal marrow will facilitate analyses of alterations during hematopoietic development that may occur in hematological disorders including paroxysmal nocturnal hemoglobinuria (PNH).

L9 ANSWER 12 OF 19 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 91102485 MEDLINE
DOCUMENT NUMBER: 91102485
TITLE: Phagocytosis of agarose beads by receptors for C3b (**CR1**) and iC3b (CR3) on alveolar macrophages from patients with sarcoidosis.
AUTHOR: Pettersen H B; Johnson E; Osen S S
CORPORATE SOURCE: Department of Internal Medicine, University of Trondheim, Norway.
SOURCE: SCANDINAVIAN JOURNAL OF IMMUNOLOGY, (1990 Dec) 32 (6) 669-77.
Journal code: UCW. ISSN: 0300-9475.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199104
AB Alveolar macrophages (AM) from sarcoidosis patients exhibit no detectable defect in their potential to synthesize the functional alternative and terminal pathway of **complement**. They also synthesize more C9 than AM from healthy controls. Various authors have suggested that sarcoid
AM have decreased phagocytic ability. In the present work we studied whether there was any difference in C3 receptor-mediated phagocytosis of serum-treated and native agarose beads by AM recovered from patients with active sarcoidosis compared with controls. AM from seven patients with active sarcoidosis and seven healthy controls were cultured under serum-free conditions for 2, 12, 24, and 48 h. We found a significantly increased **CR1** and CR3 receptor-mediated phagocytosis of native agarose beads by AM from the seven patients. **CR1** and CR3 were also detected on AM directly recovered from bronchoalveolar lavage fluid using fluorescein-**conjugated** monoclonal anti-receptor antibodies. The percentage of AM expressing CR appeared to be increased in
sarcoidosis. The reason for the enhanced phagocytosis of agarose beads by the sarcoid AM is probably the result of both increased synthesis and receptors of **complement**. Altered **complement** production and **complement** receptors may be important for the pathogenesis of this granulomatous disorder.

L9 ANSWER 13 OF 19 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 90278229 MEDLINE
DOCUMENT NUMBER: 90278229
TITLE: Relationship of chemotactic receptors for formyl peptide and C5a to **CR1**, CR3, and Fc receptors on human neutrophils.
AUTHOR: Van Epps D E; Bender J G; Simpson S J; Chenoweth D E
CORPORATE SOURCE: Applied Sciences, Baxter Healthcare Corporation, Round Lake, Illinois 60073..
CONTRACT NUMBER: CA20819 (NCI)
SOURCE: JOURNAL OF LEUKOCYTE BIOLOGY, (1990 Jun) 47 (6) 519-27.
Journal code: IWY. ISSN: 0741-5400.
PUB. COUNTRY: United States

Jou[redacted]l; Article; (JOURNAL ARTICLE)
LANGUAGE: Eng[redacted]n
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199009
AB The co-expression of C5a and formyl-methionine-leucine-phenylalanine-lysine (FMLPL) receptors with **CR1**, CR3, and Fc receptors on human neutrophils (PMN) was studied. Fluorescein-**conjugated** C5a (FL-C5a) and FMLPL (FL-FMLPL) were used to identify C5a and formyl peptide receptors. **CR1**, CR3, and Fc receptors were identified with monoclonal antibodies and a Texas red-labeled goat anti-mouse immunoglobulin second step reagent. The co-expression of chemotactic receptors with **CR1**, CR3, or Fc receptors was evaluated using two-color flow cytometry. A direct correlation between the degree of expression of receptors for FL-FMLPL and the expression of CR3, **CR1**, and Fc receptors on individual PMN was observed. In contrast, no correlation between the degree of C5a receptor expression and **CR1**, CR3, or Fc receptor expression was found. Similar results were obtained with PMN after up regulation of **CR1**, CR3, Fc, and FMLPL receptors by incubation at 37 degrees C for 10 min with or without phorbol myristate acetate. These data suggest that the expression of FMLPL, **CR1**, CR3, and Fc receptors are regulated in a similar manner, whereas C5a receptor expression is regulated independently. Furthermore, these data indicate that within a given population of PMN, a parallel exists between the degree of **CR1**, CR3, FMLPL, and Fc receptor expression on individual cells.

L9 ANSWER 14 OF 19 MEDLINE
ACCESSION NUMBER: 90164551 MEDLINE
DOCUMENT NUMBER: 90164551
TITLE: Comparison of peritoneal white blood cell parameters from continuous ambulatory peritoneal dialysis patients with a high or low incidence of peritonitis.
AUTHOR: Holmes C J; Lewis S L; Kubey W Y; Van Epps D E
CORPORATE SOURCE: Baxter Healthcare, Round Lake, IL 60073..
SOURCE: AMERICAN JOURNAL OF KIDNEY DISEASES, (1990 Mar) 15 (3) 258-64.
Journal code: 3H5. ISSN: 0272-6386.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
AB The purpose of this study was to determine if there were differences in selected dialysate white blood cells (WBC) parameters between continuous ambulatory peritoneal dialysis (CAPD) patient groups identified as having a high or low incidence of peritonitis. Parameters studied were total peritoneal WBC yield, percentage and absolute number of various WBC types, and expression of WBC receptors known to be involved in normal host defense mechanisms. WBCs were obtained from peritoneal dialysis effluents (overnight dwell), which were collected at monthly intervals for 6 to 8 months from eight CAPD patients--four with a history of high peritonitis incidence (HPI) (more than two episodes in 12 months) and four with a history of low peritonitis incidence (LPI) (no episodes in more than 24 months). Our results demonstrated that there was no significant difference in the overall mean total cell yields or absolute cell counts between the two patient groups. WBC differentials, although differing somewhat among patients, stayed quite stable over time for an individual patient and there was no significant difference between the two patient groups. Analysis of receptors on the peritoneal WBC was performed using flow cytometry and fluorescein-**conjugated** chemotactic factors (C5a and fMet-Leu-Phe-Lys), as well as monoclonal antibodies specific for Fc receptors and **complement** receptors, **CR1** (CD35) and CR3 (CD11b). Although there was a trend toward increased expression of all these receptors in the HPI patients, there was no significant difference in the fluorescence intensity of peritoneal neutrophils or macrophages that expressed these receptors between the two patient groups.(ABSTRACT

TRUNCATED AT 250 WORDS)

L9 ANSWER 15 OF 19 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 89323118 MEDLINE
DOCUMENT NUMBER: 89323118
TITLE: Translation of the human C3b/C4b receptor mRNA in a cell-free system and by Xenopus oocytes.
AUTHOR: Kumar V; Farries T; Swierkoz J; Atkinson J P
CORPORATE SOURCE: Howard Hughes Medical Institute Laboratories, Washington University School of Medicine, St. Louis, Missouri 63110.
SOURCE: BIOCHEMISTRY, (1989 May 2) 28 (9) 4040-6.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198911
AB The C3b/C4b **complement** receptor (**CR1**) is a large, single-chain integral membrane glycoprotein present on erythrocytes, leukocytes, glomerular podocytes, and splenic dendritic-reticular cells that mediates the binding of **complement**-coated particles and immune complexes. **CR1** is unusual in that it is polymorphic in size with the four allelic variants having molecular weights of 190,000, 220,000, 250,000, and 280,000 (SDS-PAGE, reducing conditions). The in vitro translation of the common (Mr 220,000) allelic variant **CR1** has been achieved by using mRNA in lysates of rabbit reticulocytes and in Xenopus oocytes. HL-60, a promyelocytic human leukemic cell line, was treated with DMSO to induce differentiation and synthesis of **CR1** . Poly(A+) RNA was purified from these cells by column chromatography on oligo(dT)-cellulose. In the rabbit reticulocyte system, no **CR1** was detected unless the translation mixture was denatured. In the presence of methylmercuric hydroxide, the **CR1** translation product, unlike most translation products, had the same molecular weight in gel electrophoresis as the high-mannose-containing pro-**CR1** and was 15-20K larger than nonglycosylated **CR1**. This suggests that a cotranslational modification of **CR1** structure occurs, probably involving a proteolytic cleavage event. When poly(A+) RNA was translated in Xenopus oocytes, **CR1** could be detected by treatment of oocytes with anti-**CR1** monoclonal antibody followed by fluorescein-**conjugated** goat anti-mouse IgG. **CR1** was diffusely distributed but preferentially localized to the vegetal surface.
The molecular weight of this product, identified in immunoprecipitates of lysates of [35S]methionine-labeled oocytes, was identical with that of **CR1** of HL-60.

L9 ANSWER 16 OF 19 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 89135006 MEDLINE
DOCUMENT NUMBER: 89135006
TITLE: B-cell restricted saporin immunotoxins: activity against B-cell lines and chronic lymphocytic leukemia cells.
AUTHOR: Bregni M; Siena S; Formosa A; Lappi D A; Martineau D; Malavasi F; Dorken B; Bonadonna G; Gianni A M
CORPORATE SOURCE: Division of Medical Oncology, Istituto Nazionale Tumori, Milano, Italy.
SOURCE: BLOOD, (1989 Feb 15) 73 (3) 753-62.
Journal code: A8G. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 198906
AB B cell-restricted immunotoxins were constructed by **conjugating** anti-B monoclonal antibodies to saporin, the major ribosome inactivating protein from the seeds of the plant Saponaria officinalis. HD37-SAP is directed against CD19, the broadest B cell-specific determinant. HD39-SAP and HD6-SAP recognize two different epitopes on the CD22 molecule, an

antigen present on the cell surface of B cells at late stages of differentiation. All three immunotoxins inhibited DNA synthesis and protein synthesis in target B lymphoma cells with a dose-related effect, in short incubation times and in the absence of potentiators. A clonogenic assay demonstrated that all immunotoxins could eliminate more than two logs of clonogenic malignant B cells with a two-hour incubation at concentrations not toxic to cells not bearing target antigens. The immunotoxin activity was evaluated by DNA synthesis inhibition in fresh B-chronic lymphocytic leukemia cells (B-CLL) stimulated to proliferate by incubation with an antibody specific for the receptor of C3b **complement** component (**CR1**) plus B cell growth factor. B-CLL cell DNA synthesis was actively inhibited by treatment at low immunotoxin concentration without need of potentiators. Immunotoxins exerted their effect also in whole blood of CLL patients under conditions achievable in vivo. We conclude that B cell-restricted immunotoxins HD37-SAP, HD39-SAP, and HD6-SAP are good candidates for in vivo therapy of B-cell malignancies.

L9 ANSWER 17 OF 19 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 89035527 MEDLINE
DOCUMENT NUMBER: 89035527
TITLE: Direct evidence for the clustered nature of **complement** receptors type 1 on the erythrocyte membrane.
AUTHOR: Paccaud J P; Carpentier J L; Schifferli J A
CORPORATE SOURCE: Department of Medicine, University Cantonal Hospital, Geneva, Switzerland.
SOURCE: JOURNAL OF IMMUNOLOGY, (1988 Dec 1) 141 (11) 3889-94. Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 198902
AB C receptors 1 (**CR1**) of human E are involved in the transport of C3b-coated immune complexes (IC) in the circulation. Many studies have suggested that the binding of IC to E is multivalent. This would require **CR1** to be clustered on the cell membrane, but no direct evidence for such clustering is available. We studied the distribution of **CR1** on human E by immunofluorescence and shadow-casting immuno-electron microscopy techniques with the use of a monoclonal anti-**CR1** antibody followed by FITC- or gold-**conjugated** second antibodies, respectively. By immunofluorescence, **CR1** appeared as small dots (clusters) on fixed and unfixed E prepared either at 4 degrees C or at 37 degrees C. In the same donor, the number of clusters varied extensively from cell to cell (e.g., 1 to 43 clusters/E for a donor with 520 **CR1**/cell), but the mean number of clusters per cell correlated significantly with the mean number of **CR1**/cell. These images contrasted with those obtained for Rhesus D (RhD) Ag used as controls (RhD Ag are known to be evenly distributed): only a faint uniform fluorescence was seen despite the presence of 10,000 antigenic sites. As determined by immunocytochemical method, more than 65% of the total gold particles were organized in clusters (2 to 15 gold particles/cluster) whether cells were prefixed or not. Quantitative determinations suggested that each gold particle corresponded to one **CR1**. The fraction of gold particles grouped into clusters of three or more receptors, the mean size of the clusters, and the maximal size of clusters correlated with the mean number of **CR1** per cell. By contrast, RhD Ag were distributed homogeneously (less than 2% gold particles in clusters). These data are the first to demonstrate the preclustered nature of **CR1** on E. Such distribution could explain the high binding efficiency of C3b-coated IC to E despite the low number of **CR1** per cell.

L9 ANSWER 18 OF 19 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 88162623 MEDLINE
DOCUMENT NUMBER: 88162623
TITLE: Immunohistochemical studies of **complement** receptor (**CR1**) in cases with normal sinus mucosa and chronic sinusitis.
AUTHOR: Miyaguchi M; Uda H; Sakai S; Kubo T; Matsunaga T
CORPORATE SOURCE: Department of Otolaryngology, Kagawa Medical School, Japan..
SOURCE: ARCHIVES OF OTO-RHINO-LARYNGOLOGY, (1988) 244 (6) 350-4. Journal code: 87K. ISSN: 0302-9530.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198806
AB The **complement** receptor (**CR1**) in the maxillary sinus mucosa of normal patients and in cases of chronic sinusitis was studied with the peroxidase-antiperoxidase, avidin-biotin peroxidase and immunofluorescent methods. **CR1** was localized on the ciliary surface and in the cytoplasm of the covering epithelium in both normal controls and the cases of chronic sinusitis. **CR1** tended to be denser in the mucosa of chronic sinusitis than in normal mucosa. **CR1**-binding capacity was also studied with the immunofluorescent method, using C3b-**conjugated** zymosan. Although **CR1** did not bind to C3b in vivo, it was found to bind to C3b in the normal maxillary mucosa when it was treated with C3b-**conjugated** zymosan. **CR1**-binding capacity could not be detected in the mucosa from cases with chronic sinusitis, indicating that **CR1** was already bound to activated C3b in these cases.

L9 ANSWER 19 OF 19 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 84089060 MEDLINE
DOCUMENT NUMBER: 84089060
TITLE: Coupling of C3b to erythrocytes by disulfide bond formation: preparation of EC3b for hemolytic and **complement** receptor assays.
AUTHOR: Lambris J D; Scheiner O; Schulz T F; Alsenz J; Dierich M P
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1983 Dec 30) 65 (3) 277-83.
Journal code: IFE. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198404
AB We describe a new method of preparing C3-coated erythrocytes by coupling C3 to thiol-activated erythrocytes. The procedure involves three steps. Firstly, sheep erythrocytes were treated with N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) to introduce 3-(2-pyridyldithio) propionyl residues into membrane proteins. Secondly, C3 was cleaved with trypsin or CoVF, Bb enzyme to obtain C3b exposing the SH group (C3b-SH). Finally, the C3b-SH was coupled to the thiol-activated erythrocytes (TA-E) through thiol/disulfide exchange to form the TA-EC3b **conjugate**. E coated with C3d was prepared by treating TA-EC3b with KSCN inactivated serum and plasmin. Studying the rosette formation between TA-EC3b or TA-EC3d and cells expressing C3b (**CR1**) and C3d (CR2) receptors and the inhibition thereof with anti-**CR1** and anti-CR2 antibodies as well as with C3-sheep E membrane protein complexes, we found that TA-EC3b and TA-EC3d bound exclusively to **CR1** and CR2, respectively. In addition, TA-EC3b like EAC1423b bound factors B and H as tested by hemolytic and direct binding assays. The advantage of TA-EC3 for **complement** receptor and hemolytic assays are the simplicity of the preparation method and the general applicability of the TA-EC3.

=> s (soluble CR1) or (soluble (2W) cr1)

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L10         132 (SOLUBLE    ) OR (SOLUBLE (2W) CR1)

=> s l10 not l9

L11         132 L10 NOT L9

=> d his


     (FILE 'HOME' ENTERED AT 13:17:52 ON 20 FEB 2001)

     FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 13:18:30 ON 20 FEB 2001
L1          3493 S CR1
L2          2441 S CR1 AND COMPLEMENT
L3             3 S L2 AND FATTY
L4             2 DUP REM L3 (1 DUPLICATE REMOVED)
L5            10 S L1 AND FATTY
L6             7 S L5 NOT L3
L7             7 DUP REM L6 (0 DUPLICATES REMOVED)
L8            36 S L2 AND CONJUG?
L9            19 DUP REM L8 (17 DUPLICATES REMOVED)
L10          132 S (SOLUBLE CR1) OR (SOLUBLE (2W) CR1)
L11          132 S L10 NOT L9

=> s soluble cr1

L12          87 SOLUBLE CR1

=> dup rem l12

PROCESSING COMPLETED FOR L12
L13           44 DUP REM L12 (43 DUPLICATES REMOVED)

=> s l13 py <1998

MISSING OPERATOR L13 PY
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l13 and py <1998

   2 FILES SEARCHED...
L14          26 L13 AND PY <1998

=> d ibib abs 1-26


L14  ANSWER 1 OF 26  MEDLINE
ACCESSION NUMBER:   1998182740     MEDLINE
DOCUMENT NUMBER:    98182740
TITLE:              Soluble complement receptor 1 (sCR1) is not as effective
as
                    cobra venom factor in the treatment of experimental
                    allergic neuritis.
AUTHOR:             Vriesendorp F J; Flynn R E; Pappolla M A; Koski C L
CORPORATE SOURCE:   Department of Neurology, University of Texas Health
Science
                    Center, Houston, USA.
SOURCE:             INTERNATIONAL JOURNAL OF NEUROSCIENCE, (1997 Dec)
                    92 (3-4) 287-98.
                    Journal code: GS4. ISSN: 0020-7454.
PUB. COUNTRY:       ENGLAND: United Kingdom
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        199807
ENTRY WEEK:         19980702
AB   To further investigate the role of complement activation in Experimental
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Allergic Neuritis (EAN), the effect of systemic complement blockade by **soluble CR1** (sCR1) was compared to complement depletion by Cobra Venom Factor (CVF) in EAN rats immunized with bovine peripheral nerve myelin. EAN rats treated with CVF (n = 10) had significantly reduced clinical scores compared to rats treated with sCR1 (n = 9) or saline (n = 10) (score: sCR1 0.66 +/- 0.7; CVF 0; saline 0.6 +/- 0.8; mean +/- SD). CVF treatment more effectively decreased inflammation and demyelination compared to sCR1 treatment which had only a partial effect (inflammation: sCR1 1.8 +/- 1.4; CVF 0.3 +/- 0.7; saline 1.9 +/- 1.2; demyelination; sCR1 1.3 +/- 1; CVF 0.1 +/- 0.6; saline 1.7 +/- 1.2). In lumbosacral nerve roots significantly less infiltrating ED1 positive macrophages and CD11bc (expressing complement receptor 3 or CR3) positive inflammatory cells were present in CVF treated EAN rats while there was a limited decrease in inflammation in the sCR1 treated animals compared to the saline treated rats (ED1: sCR1 1.4 +/- 1.2; CVF 0.5 +/- 0.6; saline 1.7 +/- 1.2; CD11bc: sCR1 1.9 +/- 1.2; CVF 0.9 +/- 1; saline 2.1 +/- 1.2). Our findings suggest that complement depletion by CVF is more effective than complement blockade by sCR1 in reducing the severity of inflammatory peripheral nerve demyelination.

L14 ANSWER 2 OF 26 MEDLINE
ACCESSION NUMBER: 1998034979 MEDLINE
DOCUMENT NUMBER: 98034979
TITLE: Neutrophil Fc gamma and complement receptors involved in binding soluble IgG immune complexes and in specific granule release induced by soluble IgG immune complexes.
AUTHOR: Voice J K; Lachmann P J
CORPORATE SOURCE: Molecular Immunopathology Unit, MRC Center, Cambridge, GB.
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, **(1997 Oct)** 27 (10) 2514-23.
Journal code: EN5. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199802
ENTRY WEEK: 19980204

AB We examined the effect of soluble IgG immune complex (IC) characteristics on the binding of IC to human neutrophils and IC-induced specific granule release of neutrophils via Fc gamma receptors (CD16 and CD32) and complement receptors (CR1 and CR3). A set of soluble IgG IC varying in size, IgG subclass, antigen epitope density and complement (C) incorporation were formed between 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP) coupled to bovine serum albumin (BSA) and chimeric mouse-human anti-NIP monoclonal antibodies (mAb) of all four IgG subclasses. High and low epitope density IC of all four IgG subclasses induced specific granule release with C, but in the absence of C only IgG1 and IgG3 IC were functionally active. The Fc gamma and C receptors responsible for IgG IC-induced specific granule release and IC binding were determined using mAb specific for the ligand binding sites of CD16, CD32 and CR3, and recombinant **soluble CR1**. Each defined IC displayed a unique pattern of receptor preference, dependent upon subclass and antigenic epitope density. IC binding and IC-induced specific granule release was not mediated by the same receptor, or combination of receptors. High and low epitope density IgG3 IC binding and induction of specific granule release was mediated predominantly via CD16. Other IC subclasses bound differently, i.e. IgG1 IC used CD16 and CR3; IgG2 and IgG4 predominantly used complement receptors; but all three induced specific granule release via CD32. In vivo these results may translate into differential activation of neutrophils by soluble IC dependent upon their characteristics, leading to subtle nuances in the etiology, pathology and control of the immune response in IC-related diseases.

L14 ANSWER 3 OF 26 MEDLINE
ACCESSION NUMBER: 97464534 MEDLINE
DOCUMENT NUMBER: 97464534
TITLE: Complement receptor type 1 (CR1, CD35) is a receptor for C1q.
AUTHOR: Klickstein L B; Barbashov S F; Liu T; Jack R M; Nicholson-Weller A
CORPORATE SOURCE: Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215, USA.
CONTRACT NUMBER: DK34028 (NIDDK)
SOURCE: IMMUNITY, **(1997 Sep)** 7 (3) 345-55.
Journal code: CCF. ISSN: 1074-7613.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY WEEK: 19980104
AB Molecular definition of the cellular receptor for the collagen domain of C1q has been elusive. We now report that C1q binds specifically to human CR1 (CD35), the leukocyte C3b/C4b receptor, and the receptor on erythrocytes for opsonized immune complexes. Biotinylated or radioiodinated C1q (*C1q) bound specifically to transfected K562 cells expressing cell surface CR1 and to immobilized recombinant **soluble CR1** (rsCR1). *C1q binding to rsCR1 was completely inhibited by unlabeled C1q and the collagen domain of C1q and was partially inhibited by C3b dimers. Kinetic analysis in physiologic saline of the interaction of unlabeled C1q with immobilized rsCR1 using surface plasmon resonance yielded an apparent equilibrium dissociation constant (K[eq2]) of 3.9 nM. Thus, CR1 is a cellular C1q receptor that recognizes all three complement opsonins, namely, C1q, C3b, and C4b.

L14 ANSWER 4 OF 26 MEDLINE
ACCESSION NUMBER: 97331588 MEDLINE
DOCUMENT NUMBER: 97331588
TITLE: Biocompatibility: complement as mediator of tissue damage and as indicator of incompatibility.
AUTHOR: Mollnes T E
CORPORATE SOURCE: Department of Immunology and Transfusion Medicine, Nordland
Central Hospital, Bodo, Norway.. tomeirik@fagmed.uit.no
SOURCE: EXPERIMENTAL AND CLINICAL IMMUNOGENETICS, **(1997)** 14 (1) 24-9. Ref: 26
Journal code: AOK. ISSN: 0254-9670.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY WEEK: 19971001
AB The fully biocompatible surface is the host's own intact endothelium. Blood contact with a damaged or foreign endothelium, or with an artificial surface, will lead to a certain degree of activation of the defense systems. Complement is one of these systems which has a unique property to distinguish between 'self' and 'non-self'. Recent data using complement-specific inhibitors like **soluble CR1** and monoclonal antibodies to C5 have shown that complement is not only associated with, but in fact contributes to, the whole body inflammatory reaction seen as a complication to cardiopulmonary bypass (artificial surfaces) and is responsible for the hyperacute rejection of xenografts (foreign endothelium). Complement activation, as measured by assays specific for neoepitopes exposed in the activation products, is a sensitive indicator of bioincompatibility. These assays have been increasingly important after a causal link between complement activation

and tissue damage [redacted] demonstrated. Efforts have been made to improve the biocompatibility of artificial surfaces, including coating with heparin. This procedure not only improves coagulation compatibility, but also markedly reduces complement activation. Models to study complement compatibility in vitro and in vivo are described, and recommended complement activation assays reviewed.

L14 ANSWER 5 OF 26 MEDLINE
ACCESSION NUMBER: 97226231 MEDLINE
DOCUMENT NUMBER: 97226231
TITLE: CR1, CD35 in synovial fluid from patients with inflammatory joint diseases.
AUTHOR: Sadallah S; Lach E; Lutz H U; Schwarz S; Guerne P A; Schifferli J A
CORPORATE SOURCE: University Hospital, Basel, Switzerland.
SOURCE: ARTHRITIS AND RHEUMATISM, **(1997 Mar)** 40 (3) 520-6.
Journal code: 90M. ISSN: 0004-3591.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199706
ENTRY WEEK: 19970601
AB OBJECTIVE: To investigate synovial fluid (SF) for the presence of CR1 and to study its relationship to SF leukocytes and to serum levels of **soluble CR1** (sCR1) in patients with rheumatic diseases.
METHODS: Synovial fluids were collected from 35 patients with rheumatoid arthritis (RA) and 26 patients with other inflammatory joint diseases. Total CR1 in the SF and serum were measured with a sandwich enzyme-linked immunosorbent assay (ELISA) that recognized both soluble and transmembrane forms of CR1. The characteristics of CR1 in SF were analyzed by ultracentrifugation and by a second ELISA specific for transmembrane CR1.
RESULTS: CR1 was found in all SF samples tested (range 5-281 ng/ml). SF CR1 was higher in patients with RA (mean +/- SD 81 +/- 66 ng/ml) than in those with other inflammatory joint diseases (31.8 +/- 23.8 ng/ml) ($P < 0.001$). Serum sCR1 was not significantly increased in the patients compared with the normal subjects. There was no correlation between serum sCR1 and SF CR1. In 44% of the patients, the SF CR1 level was higher than the serum sCR1 level. A fraction (30-80%) of SF CR1 was pelleted by ultracentrifugation and, unlike serum sCR1, it reacted in an ELISA specific for transmembrane CR1. Thus, SF contained 2 forms of CR1: a membrane-associated and a soluble form, which was confirmed by sucrose density-gradient ultracentrifugation. SF CR1 levels correlated directly with the number of SF total leukocytes and polymorphonuclear leukocytes (PMN). These 2 forms of CR1 were also found in the supernatant of in vitro-activated PMN from normal subjects. SF CR1 exhibited the capacity to act as a cofactor for the factor I degradation of C3b. CONCLUSION: CR1 is found in the SF of patients with joint inflammation. The data suggest that SF CR1 originates from the infiltrating leukocytes, which shed both a soluble and a membrane-associated form. Whether SF CR1 participates in the local regulation of complement activation remains to be examined.

L14 ANSWER 6 OF 26 MEDLINE
ACCESSION NUMBER: 96344058 MEDLINE
DOCUMENT NUMBER: 96344058
TITLE: Recombinant soluble human complement receptor type 1 inhibits antisperm antibody- and neutrophil-mediated injury to human sperm.
AUTHOR: D'Cruz O J; Toth C A; Haas G G Jr
CORPORATE SOURCE: Department of Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City 73190, USA..

odc[redacted]@aol.com
SOURCE: BIOL[redacted] OF REPRODUCTION, **(1996 Jun)** 5[redacted]6) 1217-28.
Journal code: A3W. ISSN: 0006-3363.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701

AB The pathogenesis of antisperm antibody (ASA)-mediated infertility is postulated to be related in part to complement (C)-dependent sperm dysfunction in the female genital tract. We have previously demonstrated that C can be involved in ASA-mediated sperm injury by the deposition of activated C3 fragments and the assembly of terminal membrane attack complex (C5b-9) leading to C3-mediated sperm binding to neutrophils or C5b-9-mediated sperm motility loss. This study evaluated the protective effect of recombinant soluble C receptor type 1 (sCR1) on ASA-and C-mediated neutrophil/sperm interaction, neutrophil aggregation, and sperm motility loss. Motile sperm with or without neutrophils were incubated in the presence of 10% C-fixing ASA+ serum or ASA- control sera in the presence or absence of sCR1. After defined incubation periods, the following neutrophil and sperm parameters were evaluated: 1) neutrophil aggregation (by the flow cytometric pulse processing method), 2) sperm phagocytosis (by light microscopy), 3) the deposition of C3 cleavage fragments (C3b, iC3b, and C3d) on motile sperm (by immunofluorescence flow cytometry), and 4) the relation between sperm motility loss and sperm-bound C3d. Only the coincubation of neutrophils with sperm in the presence of C-fixing ASA+ sera resulted in marked neutrophil aggregation (20.5 +/- 0.26% vs. 2.4% +/- 1.6; $p < 0.0001$) and a concomitant increase in neutrophils containing ingested sperm (71 +/- 5.8% vs. 3.5%; $p < 0.0001$). **Soluble CR1** inhibited ASA- and C-mediated neutrophil aggregation by 46% and sperm phagocytosis by 57%. Motile sperm incubated with C-fixing ASA- sera showed a time-dependent increase in the binding of C3 fragments as detected by flow cytometry using anti-iC3b neoantigen, anti-C3c, and anti-C3d monoclonal antibodies (mAbs). A negative correlation ($r2 = -0.930$; $p < 0.001$) was found between the increase in sperm-associated C3d fluorescence and the percentage motile sperm in the presence of ASA- sera. **Soluble CR1** (200 micrograms/ml) maximally inhibited the binding of anti-C3b, anti-C3c, and anti-C3d mAbs to sperm by 96%, 83%, and 72%, respectively. Thus, sCR1 abrogated the binding of C3 fragments to human sperm and fully protected sperm from C5b-9-mediated sperm immobilization. These findings suggested the therapeutic potential of sCR1 as an intravaginal pharmacophore to prevent C-dependent sperm dysfunction and related inflammatory events in the female genital tract.

L14 ANSWER 7 OF 26 MEDLINE
ACCESSION NUMBER: 96269510 MEDLINE
DOCUMENT NUMBER: 96269510
TITLE: Neutralization of Knops system antibodies using soluble complement receptor 1.
AUTHOR: Moulds J M; Rowe K E
CORPORATE SOURCE: Department of Internal Medicine, University of Texas-Houston Medical School, USA.
SOURCE: TRANSFUSION, **(1996 Jun)** 36 (6) 517-20.
Journal code: WDN. ISSN: 0041-1132.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610

AB BACKGROUND: Antibodies of the Knops system have been referred to as nonneutralizable because they cannot be inhibited with serum, saliva, or urine. Because the Knops system antigens have been located on complement receptor 1 (CR1), the question of whether the antibodies could be neutralized with **soluble CR1** (sCR1) produced by recombinant DNA techniques was studied. STUDY DESIGN AND METHODS: First,

radiolabeled immunoprecipitation techniques were used to test sCR1 for the expression of the high-incidence Knops system antigens. Then, a total of 45 antibodies were neutralized with sCR1, including the following: one each of anti-Cr(a), -Dr(a), -Do(b), -Hy, -Ge, -Jr(a), -Sc1, -Jk(a), -Cs(a), and -Kp(b); two each of anti-Lu(b), -Yt(a), and -JMH; three each of anti-McC(a), -Rg, and -Sl(a); and four each of anti-Ch, -Kn(a), -Yk(a), -Kn/McC. In addition, two examples of anti-Kn(a) + K, one example of anti-Sl(a) + K + Fy(a), and one example of anti-Yk(a) + E were tested. The sCR1 was added to each test serum and 6-percent albumin was added to the control; this was followed by neutralization incubation for 5 minutes at 25 degrees C. The antibody samples were then tested by a low-ionic-strength solution, anti-human globulin technique. RESULTS: The sCR1 expressed Kn(a), McC(a), Sl,a and Yk(a). All Knops system antibodies (n = 22) were neutralized by the sCR1, but none of the other 23 alloantibodies decreased in reactivity. The samples containing antibodies of two specificities showed inhibition of the Knops system antibody but not of the second antibody. CONCLUSION: This neutralization method, in which recombinant protein is used, provides an expedient and definitive method of identifying Knops system antibodies.

L14 ANSWER 8 OF 26 MEDLINE
ACCESSION NUMBER: 96106489 MEDLINE
DOCUMENT NUMBER: 96106489
TITLE: Monoclonal antibodies directed against human C5 and C8 block complement-mediated damage of xenogeneic cells and organs.
AUTHOR: Rollins S A; Matis L A; Springhorn J P; Setter E; Wolff D W
CORPORATE SOURCE: Department of Immunobiology, Alexion Pharmaceuticals, Inc., New Haven, Connecticut 06511, USA.
SOURCE: TRANSPLANTATION, **(1995 Dec 15)** 60 (11) 1284-92.
Journal code: WEJ. ISSN: 0041-1337.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199603

AB The hyperacute rejection (HAR) of xenotransplanted organs is initiated by the deposition of natural antibodies on donor endothelium followed by the activation of the recipient complement system, which rapidly destroys the graft. Studies of the role of activated complement in HAR have suggested that natural antibody as well as early (C3a, C3b) and late (C5a, C5b-9) activated complement components may contribute to cell activation and damage. Attenuation of HAR has been achieved by blockade of C3 activation with **soluble CR1** or consumptive depletion of complement with cobra venom factor; however, similar studies using specific inhibitors of terminal complement components have not been described. To address the contribution of C5a and the membrane attack complex (C5b-9, MAC) to complement-mediated xenogeneic cell and organ damage, we utilized functionally blocking monoclonal antibodies directed against the human terminal complement components C5 and C8. Our data show that both anti-C5 and anti-C8 mAbs protect porcine aortic endothelial cells from membrane damage mediated by human C5b-9. Additionally, both the anti-C5 and anti-C8 mAbs blocked complement-mediated generation of membrane prothrombinase activity on porcine aortic endothelial cells challenged with human serum. To test the ability of these antibodies to attenuate antibody and complement-mediated damage of xenogeneic organs, an ex vivo model was developed wherein isolated rat hearts were perfused with human serum in the presence or absence of the anti-C5 and anti-C8 mAbs. Our data demonstrate that mAbs directed against human C5 and C8 prevented organ damage by human serum complement and suggest that these molecules may serve as potent inhibitors of HAR.

L14 ANSWER 9 OF 26 MEDLINE
ACCESSION NUMBER: 96096455 MEDLINE
DOCUMENT NUMBER: 96096455
TITLE: Complement-mediated regulation of tissue factor activity in endothelium.
AUTHOR: Saadi S; Holzknecht R A; Patte C P; Stern D M; Platt J L
CORPORATE SOURCE: Department of Surgery, Duke University, Durham, North Carolina 27710, USA.
CONTRACT NUMBER: HL-46810 (NHLBI)
HL-52297 (NHLBI)
HL-50985 (NHLBI)
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, **(1995 Dec 1)** 182 (6) 1807-14.
Journal code: I2V. ISSN: 0022-1007.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199603
AB Inflammation and immunity may be associated with endothelial cell (EC) injury and thrombus formation. We explored the mechanisms through which a humoral immune response directed against the endothelium might promote coagulation. Using the interaction of anti-EC antibodies and complement (C) with cultured EC as a model, we studied the expression and function of tissue factor, a cofactor for factor VIIa-mediated conversion of factor X to Xa. Exposure of EC to anti-EC antibodies and C in sublytic amounts stimulated the synthesis of tissue factor over a period of 16-42 h. Cell surface expression of tissue factor activity required activation of C and assembly of the membrane attack complex, because expression was inhibited by **soluble CR1** and was not detected in the absence of C8. Elaboration of tissue factor messenger RNA was observed over a period of 8-30 h and required protein synthesis. Expression of tissue factor was not a direct consequence of the action of C on the EC but was a secondary response that required as an intermediate step the release of interleukin 1 alpha, an early product of the EC response to C activation. These findings suggest that, after the assembly of membrane attack complex on EC, the production of tissue factor and initiation of coagulation in a blood vessel depend on the production of interleukin 1 alpha and on its availability to stimulate affected EC.

L14 ANSWER 10 OF 26 MEDLINE
ACCESSION NUMBER: 96091966 MEDLINE
DOCUMENT NUMBER: 96091966
TITLE: [Hemolytic anemia, myocardial infarction and xenotransplantation: what do they have in common?]. Anemie hemolytique, infarctus du myocarde et xenotransplantation: quel point commun?.
AUTHOR: Schifferli J A
CORPORATE SOURCE: Medizinische Universitatsklinik B, Kantonsspital Basel.
SOURCE: SCHWEIZERISCHE MEDIZINISCHE WOCHENSCHRIFT. JOURNAL SUISSE DE MEDECINE, **(1995 Nov 18)** 125 (46) 2217-25.
Journal code: UEI. ISSN: 0036-7672.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: French
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
AB Complement is activated by antibodies, by necrotic cells and by the many surfaces which do not inhibit the amplification loop of the alternative pathway. Examples of such activation include hyperacute rejection in xenotransplantation models (antibody), myocardial infarction (necrotic cells) and paroxysmal nocturnal hemoglobinuria (PNH: lack of complement control on abnormal erythrocytes and platelets). Over the past decade many of the complement regulatory proteins have been described, analyzed and produced in recombinant forms. One of these proteins is complement

receptor 1 (CR1, CD[illegible]), which in soluble form is capable of inhibiting complement activation in vivo. In many experimental models, **soluble CR1** has been shown to reduce antibody-mediated damage, diminish inflammation at the time of cell necrosis and inhibit lysis of PNH cells. For the first time it will be possible to inhibit complement activation in humans.

L14 ANSWER 11 OF 26 MEDLINE
ACCESSION NUMBER: 95202791 MEDLINE
DOCUMENT NUMBER: 95202791
TITLE: Complement-mediated loss of endothelium-dependent relaxation of porcine coronary arteries. Role of the terminal membrane attack complex.
AUTHOR: Stahl G L; Reenstra W R; Frendl G
CORPORATE SOURCE: Brigham and Women's Hospital, Department of Anesthesia, Boston, MA 02115..
SOURCE: CIRCULATION RESEARCH, **(1995 Apr)** 76 (4) 575-83.
Journal code: DAJ. ISSN: 0009-7330.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
AB Reperfusion of the ischemic myocardium results in the loss of endothelium-dependent relaxation. We have shown recently that the alternate complement pathway is activated immediately on reperfusion of the ischemic porcine myocardium. We hypothesized that complement activation directly attenuates endothelium-dependent relaxation of porcine coronary arteries. Bradykinin (BK) or substance P concentration-dependently relaxed precontracted (U46619, 50 nmol/L) left anterior descending coronary artery (LAD) rings in vitro. Addition of zymosan to human (10%) or porcine (10%) serum for 30 minutes significantly ($P < 0.05$) increased the EC50 of BK-induced LAD relaxation from 4 +/- 1 to 418 +/- 159 nmol/L (n = 8) and from 9 +/- 3 to 281 +/- 132 nmol/L (n = 7), respectively. Similarly, addition of zymosan to 10% human serum (HS) for 30 minutes increased the EC50 of substance P-induced LAD relaxation from 0.4 +/- 0.1 to 30 +/- 14 nmol/L (n = 9, $P < .05$). Basal release of nitric oxide was reduced significantly in LAD rings exposed to zymosan-activated HS compared with HS alone. Addition of **soluble CR1** (sCR1, 10 nmol/L) to zymosan-activated HS preserved BK-induced relaxation (EC50) of the LAD rings (control, 4 +/- 1 nmol/L; sCR1 + zymosan+serum, 2 +/- 1 nmol/L; n = 6). Zymosan-activated C8-depleted HS (10%) did not attenuate the EC50 of BK-induced coronary artery relaxation (3 +/- 1 to 3 +/- 1 nmol/L, n = 7, P = NS). Zymosan-activated C8-depleted HS plus C8 (6 micrograms/mL) increased the EC50 of BK-induced coronary artery relaxation from 4 +/- 1 to 423 +/- 141 nmol/L (n = 12, $P < .05$). We have further demonstrated that C5b-9 complexes can be found on the luminal surface of LAD endothelial cells after 5 minutes of exposure to zymosan-activated HS by using C5b-9 reactive monoclonal antibody fluorescent immunohistochemistry and confocal microscopy.(ABSTRACT TRUNCATED AT 250 WORDS)

L14 ANSWER 12 OF 26 MEDLINE
ACCESSION NUMBER: 95045920 MEDLINE
DOCUMENT NUMBER: 95045920
TITLE: Soluble complement receptor type 1 (CD35) is released from leukocytes by surface cleavage.
AUTHOR: Danielsson C; Pascual M; French L; Steiger G; Schifferli J A
CORPORATE SOURCE: Laboratory of Immunonephrology, Medizinische Klinik B, Department Innere Medizin, Kantonsspital Basel, Switzerland..
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, **(1994 Nov)** 24 (11) 2725-31.
Journal code: EN5. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199502
AB The soluble form of complement receptor type 1 in human plasma (sCR1) might correspond to the shedding of the receptor by proteolytic cleavage at the cell surface. A new enzyme-linked immunosorbent assay (ELISA) was established to specifically measure membrane-bound CR1 using a rabbit polyclonal antibody against a 19-amino acid peptide corresponding to the C-terminal sequence of the intracellular domain of CR1 (mCR1-ELISA). This ELISA measured CR1 from solubilized erythrocyte membranes, polymorphonuclear leukocytes (PMN), a B lymphocyte cell line and renal podocyte-derived urinary vesicles in a dose-dependent manner. In contrast, and similarly to recombinant **soluble CR1** which lacks the intracellular domain of CR1, plasmatic sCR1 was not recognized, suggesting that sCR1 corresponds to an extracellular fragment of whole CR1. In vitro, PMN were shown to release a soluble form of CR1 which was also not recognized in the mCR1-ELISA, and whose size was smaller (5 kDa) than the CR1 of PMN cell membranes. The release of **soluble CR1** was highest for PMN and HL60 cells, followed by U937 cells and three different B lymphocyte cell lines, whereas T lymphocyte cell lines did not release **soluble CR1**. The levels of CR1 gene expression were also higher in PMN compared to remaining blood leukocytes and the different cell lines tested above. Incubation of PMN with formyl-methionyl-leucyl-phenylalanine, tumor necrosis factor-alpha or lipopolysaccharide accelerated the release of **soluble CR1**, and incubation with granulocyte/macrophage colony-stimulating factor resulted in sustained CR1 gene expression and higher total **soluble CR1** release. Our results suggest that **soluble CR1** is produced by cleavage of cell surface CR1, and that a large fraction of human plasma sCR1 is cleaved from PMN. The release of sCR1 by leukocytes may play a role in the control of complement activation at sites of inflammation.

L14 ANSWER 13 OF 26 MEDLINE
ACCESSION NUMBER: 94011178 MEDLINE
DOCUMENT NUMBER: 94011178
TITLE: Presence of serum modulates expression of complement receptor type 1 (CR1) on human granulocytes after quartz exposure.
AUTHOR: Lundahl J; Eklund A; Hed J; Tornling G; Vitas M
CORPORATE SOURCE: Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden.
SOURCE: INFLAMMATION, **(1993 Aug)** 17 (4) 511-9.
Journal code: GM0. ISSN: 0360-3997.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
AB We have investigated the interaction between granulocytes and quartz with respect to the expression of complement receptor type 1 (CR1) and the presence of normal human serum (NHS). Quartz down-regulates selectively CR1 on activated granulocytes. This down-regulation is abolished in the presence of both NHS and heat-inactivated NHS (NHS56) but not human albumin. When quartz was preincubated with NHS (quartz-NHS) before exposure to activated granulocytes, a down-regulating effect was observed in contrast to preincubation with NHS56, which did not induce a down-regulation. Preincubation with cytochalasin B reduced the down-regulation of quartz-NHS, indicating a cytoskeleton-dependent internalization of the receptor. The serine protease inhibitor PMSF partly reduced this down-regulation. Our results indicate that the presence of NHS in the alveolar space influences the interaction between quartz and recruited granulocytes with respect to CR1 expression. Since CR1 is an important opsonin receptor and **soluble CR1** can modulate the inflammatory response, this may be of importance in the

inflammation and fibrosing process induced by quartz in the alveolar space
and lung interstitium.

L14 ANSWER 14 OF 26 MEDLINE
ACCESSION NUMBER: 93380510 MEDLINE
DOCUMENT NUMBER: 93380510
TITLE: Construction, expression and functional analysis of a glycolipid-linked form of CR1.
AUTHOR: Clissold P M; Ebling H J; Lachmann P J
CORPORATE SOURCE: Molecular Immunopathology Unit, Medical Research Council Centre, Cambridge, GB.
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, **(1993 Sep)** 23 (9) 2346-52.
Journal code: EN5. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-Y00816; GENBANK-M11749
ENTRY MONTH: 199312
AB By genetic engineering of human CR1 cDNA and its stable transfection into cells we have produced a cell line which expresses CR1 anchored to the cell surface by a glycolipid anchor. The glycosyl-phosphatidylinositol (GPI)-CR1 protects cells intrinsically from damage mediated by complement activated through the classical pathway. Cell surface GPI-CR1 is more efficient on a molar basis than **soluble CR1** in the assay, but extrinsic protection of other cells was not obtained. **Soluble CR1**-protected cells extrinsically in the assay but was required at nearly ten fold higher amounts than the intrinsic protection conferred by GPI-anchored CR1. Additionally, GPI-CR1 was shown to act as a co-factor to Factor I in the generation of C3c from iC3b. Since GPI-anchored proteins can incorporate spontaneously into the membranes of living cells by virtue of their lipid tails, the isolated GPI-CR1 will be used to introduce CR1 on to the surfaces of many different types of cell so that its role in immunity can be further investigated.

L14 ANSWER 15 OF 26 MEDLINE
ACCESSION NUMBER: 93329152 MEDLINE
DOCUMENT NUMBER: 93329152
TITLE: Circulating **soluble CR1** (CD35). Serum levels in diseases and evidence for its release by human leukocytes.
AUTHOR: Pascual M; Duchosal M A; Steiger G; Giostra E; Pech`ere A; Paccaud J P; Danielsson C; Schifferli J A
CORPORATE SOURCE: Laboratory of Immunonephrology, Centre Medical Universitaire, Geneva, Switzerland..
SOURCE: JOURNAL OF IMMUNOLOGY, **(1993 Aug 1)** 151 (3) 1702-11.
Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199310
AB C receptor type 1 (CR1, CD35) is present in a soluble form in plasma (sCR1). **Soluble CR1** was measured with a specific ELISA assay in normal individuals and in patients with different diseases. The mean serum concentration of sCR1 in 31 normal donors was 31.4 +/- 7.8 ng/ml, and was identical in plasma. An increase in sCR1 was observed in 36 patients with end-stage renal failure on dialysis (54.8 +/- 11.7 ng/ml, $p < 0.0001$), and in 22 patients with liver cirrhosis (158.3 +/- 49.9 ng/ml, $p < 0.0001$). The mean sCR1 levels dropped from 181 +/- 62.7 to 52.1 +/- 24.0 ng/ml ($p < 0.001$) in nine patients who underwent liver transplantation, and was 33.5 +/- 7.3 in 10 patients with functioning renal grafts, indicating that the increase in sCR1 was reversible.

Soluble CR1 was elevated in some hematologic malignancies (> 47 [redacted] ml), which included B cell lym[redacted]ma (12/19 patients), Hodgkin's lymphoma (4/4), and chronic myeloproliferative syndromes (4/5). By contrast, no increase was observed in acute myeloid or lymphoblastic leukemia (10) or myeloma (5). In two patients with chronic myeloproliferative syndromes, sCR1 decreased rapidly after chemotherapy. The mean concentration of sCR1 was not significantly modified in 181 HIV-infected patients at various stages of the disease (34.8 +/- 14.4 ng/ml), and in 13 patients with active SLE (38.3 +/- 19.6 ng/ml), although in both groups the number of CR1 was diminished on E. There was a weak but significant correlation between sCR1 and CR1 per E in HIV infection and SLE ($r = 0.39$, $p < 0.0001$, and $r = 0.60$, $p < 0.03$ respectively). In vitro, monocytes, lymphocytes, and neutrophils were found to release sCR1 into culture supernatants. In vivo, sCR1 was detected in the serum of SCID mice populated with human peripheral blood leukocytes. The sCR1 levels correlated with those of human IgG ($r = 0.97$, $p < 0.0001$), suggesting synthesis of sCR1 by the transferred lymphocytes. The mechanisms underlining the increased levels of sCR1 and its biologic consequences remain to be defined.

L14 ANSWER 16 OF 26 MEDLINE
ACCESSION NUMBER: 93139495 MEDLINE
DOCUMENT NUMBER: 93139495
TITLE: Soluble complement receptor type 1 prevents human complement-mediated damage of the rabbit isolated heart.
AUTHOR: Homeister J W; Satoh P S; Kilgore K S; Lucchesi B R
CORPORATE SOURCE: University of Michigan Medical School, Department of Pharmacology, Ann Arbor 48109-0626..
CONTRACT NUMBER: HL-19782-14 (NHLBI)
SOURCE: JOURNAL OF IMMUNOLOGY, **(1993 Feb 1)** 150 (3) 1055-64.
Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199304
AB The purpose of this study was to determine if recombinant human **soluble CR1** (sCR1) could prevent tissue damage associated with the activation of human complement. Directly mediated human complement-dependent myocardial injury was induced in the rabbit isolated heart perfused with a Krebs-Henseleit buffer containing 6% human plasma. There were three study groups: 1) 6% heat-inactivated human plasma (control); 2) 6% normal human plasma (NHP); or 3) 6% normal human plasma + 20 nM sCR1 (NHP + sCR1). Recorded functional parameters of the control group remained stable throughout the duration of the 70-min protocol. Complement activation in hearts perfused with 6% NHP increased the diastolic pressure; decreased developed pressure; and increased coronary perfusion pressure. These alterations were accompanied by a decrease in the maximum positive and negative dP/dt. Complement activation also increased cardiac muscle lymphatic fluid flow rate. The changes were greatest between 20 and 40 min, but persisted for the duration of the protocol. sCR1 (20 nM) in the perfusate containing 6% NHP prevented the complement-mediated alterations in the systolic, developed, and coronary perfusion pressures. sCR1 prevented the decrement in the positive and negative dP/dt, and the increase in the lymphatic fluid flow rate. Values for each of these parameters in hearts perfused with 6% NHP + sCR1 were not altered from those of controls at any time point in the protocol. Ultrastructural changes were present in tissues perfused with 6% NHP along with immunohistochemical evidence for presence of the terminal C5b-9

complex. sCR1 prevented the ultrastructural changes and the formation of the terminal complex. sCR1 offers significant protection against the cytolytic effects resulting from activation of the human complement system.

L14 ANSWER 17 OF 26 MEDLINE
ACCESSION NUMBER: 92166387 MEDLINE
DOCUMENT NUMBER: 92166387
TITLE: Protective effects of **soluble CR1** in complement- and neutrophil-mediated tissue injury.
AUTHOR: Mulligan M S; Yeh C G; Rudolph A R; Ward P A
CORPORATE SOURCE: Department of Pathology, University of Michigan Medical School, Ann Arbor 48109..
CONTRACT NUMBER: HL-31963 (NHLBI)
HL-40526 (NHLBI)
GM-29507 (NIGMS)
+
SOURCE: JOURNAL OF IMMUNOLOGY, **(1992 Mar 1)** 148 (5) 1479-85.
Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199206
AB Complement activation is an important step for triggering of acute inflammatory reactions. Soluble human recombinant complement receptor type 1 (sCR1) blocks complement activation by both classical and alternative pathways. In addition to glycogen-induced peritonitis, three models of complement-dependent acute inflammatory injury have been used to assess the protective effects of sCR1: lung and dermal injury after intraalveolar or intradermal deposition of IgG immune complexes; acute lung injury resulting from intravascular activation of complement after the i.v. injection of cobra venom factor; and acute skin and lung injury (at 4 h) after thermal trauma involving 25 to 30% total body surface area. Vascular injury was quantified by increases in vascular permeability, hemorrhage, neutrophil infiltration, and, as indicated, tissue water content. Intravenous infusion of sCR1 reduced lung and dermal vascular injury in all models studied. In glycogen-induced peritoneal exudates sCR1-reduced neutrophil accumulation by 79%. In animals undergoing IgG immune complex-induced alveolitis, sCR1 treatment reduced vascular permeability and hemorrhage by 72 and 71%, respectively, and tissue accumulation of neutrophils was reduced by 68%. After cobra venom factor injection, sCR1 reduced increases in lung vascular permeability by 67%, hemorrhage by 73%, and lung myeloperoxidase content by 55%. Four hours after thermal injury of skin, sCR1-treated animals demonstrated significant protection against lung injury; increases in vascular permeability and hemorrhage were reduced by 45 and 46%, respectively, and myeloperoxidase content was lowered by 39%. In thermal injury of the skin, sCR1 injection reduced dermal vascular permeability by 25% at 1 h (p = NS) and 44% at 4 h. Water content in skin biopsies was also decreased. There was a dose-response relationship between the amount of sCR1 infused and the extent of protection in each of the injury models. These data demonstrate that sCR1 offers significant protection against complement-dependent tissue injury in the animal models studied and that the protective effects are related to reduced neutrophil content.

L14 ANSWER 18 OF 26 MEDLINE
ACCESSION NUMBER: 92087379 MEDLINE
DOCUMENT NUMBER: 92087379
TITLE: Recombinant **soluble CR1** suppressed complement activation, inflammation, and necrosis associated with reperfusion of ischemic myocardium.
AUTHOR: Weisman H F; Bartow T; Leppo M K; Boyle M P; Marsh H C Jr;

Car[redacted] G R; Roux K H; Weisfeldt M L; [redacted]aron D T
CORPORATE SOURCE: Dep[redacted]ment of Medicine, Johns Hopkin[redacted]iversity School of Medicine, Baltimore, MD 21205.
SOURCE: TRANSACTIONS OF THE ASSOCIATION OF AMERICAN PHYSICIANS, **(1990)** 103 64-72.
Journal code: W5P. ISSN: 0066-9458.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203

AB In summary, conversion of wild-type CR1 to a soluble form (sCR1) creates a potent inhibitor of complement activation by both the classical and alternative pathways by inhibiting the C3/C5 convertases. In the rat reperfusion infarct model, sCR1 significantly suppresses complement activation at the endothelial surface of capillaries and venules. This suppression of complement activation is accompanied by reduced accumulation of leukocytes within the infarct zone, perhaps because of reduction of the generation of C5a, which promotes expression of leukocyte adhesion receptors and leukocyte chemotaxis. In addition, formation of the C5b-9 attack complex, which may contribute to direct endothelial injury, was suppressed by sCR1. The inhibition of complement activation and leukocyte infiltration by sCR1 explains the observed significant reduction in myocardial necrosis after ischemia and reperfusion. These studies have identified sCR1 as a potential agent for therapeutic intervention in diseases associated with complement-dependent tissue injury.

L14 ANSWER 19 OF 26 MEDLINE
ACCESSION NUMBER: 91199445 MEDLINE
DOCUMENT NUMBER: 91199445
TITLE: Cellular origins of serum complement receptor type 2 in normal individuals and in hypogammaglobulinaemia.
AUTHOR: Ling N; Hansel T; Richardson P; Brown B
CORPORATE SOURCE: Department of Immunology, University of Birmingham, England..
SOURCE: CLINICAL AND EXPERIMENTAL IMMUNOLOGY, **(1991 Apr)** 84 (1) 16-22.
Journal code: DD7. ISSN: 0009-9104.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199107

AB A soluble form of complement receptor 2 (sCR2) is found in normal human serum. Amounts present are about 30-90 ng/ml, which is of the same order as reported for **soluble CR1**. Although B cells express surface CR2 and are the main peripheral blood source of sCR2 they do not appear to be the major tissue source of serum sCR2. Serum levels of sCR2 of patients with hypogammaglobulinaemia were not significantly different from those of normal individuals even in the case of two brothers with Bruton's X-linked agammaglobulinaemia (XLA) lacking (CD19+) B cells. On gel filtration through Sephacryl S-300 the sCR2 from XLA serum behaved exactly like sCR2 from normal serum or sCR2 affinity purified from cell supernates of a B lymphoblastoid line or from the T-ALL line MOLT-4. In all cases a single peak appeared at the same point in the chromatogram. Possible alternative sources of serum sCR2 are follicular dendritic cells (FDC) which are known to express CR2 strongly and T6+ lymphocytes within the thymus. Peripheral T cells from adults have not been reported to express CR2. However, investigation showed that cells from the Bruton's XLA cases produced small amounts of sCR2 in culture and although no CD21 was detected on the surface of the mononuclear cells by flow cytometry, the more sensitive direct antibody rosette test readily detected CD21. Further studies showed that non-B cells from control samples of cord blood or blood of young children also weakly expressed CD21.

L14 ANSWER 20 OF 26 M NE
ACCESSION NUMBER: 85159057 MEDLINE
DOCUMENT NUMBER: 85159057
TITLE: Characterization of a soluble form of the C3b/C4b receptor (CR1) in human plasma.
AUTHOR: Yoon S H; Fearon D T
CONTRACT NUMBER: AI-07712 (NIAID)
AI-10356 (NIAID)
AI-17917 (NIAID)
+
SOURCE: JOURNAL OF IMMUNOLOGY, **(1985 May)** 134 (5) 3332-8.
Journal code: IFB. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 198507
AB A radioimmunoassay with the use of soluble 125I-Fab monoclonal anti-CR1 and rabbit IgG anti-CR1 bound to Staphylococcus aureus particles was employed to detect and quantitate CR1 antigen in human plasma. Among 16 normal individuals the concentration of **soluble CR1** in plasma ranged from 13 to 81 ng/ml, and a similar range of concentration was found in plasma from 15 patients having systemic lupus erythematosus (SLE). The amount of plasma CR1 in normal donors, but not in SLE patients, significantly correlated with the number of CR1 sites on erythrocytes (r = 0.90, p less than 0.001), and was 7.1% of the amount of receptor that was present on erythrocytes in blood. The concentration of **soluble CR1** was not diminished by ultracentrifugation or ultrafiltration of plasma, was not affected by various modes of anti-coagulation or even by clotting of blood, and did not change during incubation of blood at 4 degrees C for up to 4 hr. On sucrose density gradient ultracentrifugation of plasma the CR1 was distributed as a broad peak that overlapped the plasma protein profile. The Mr of plasma CR1 was identical to that of erythrocyte CR1 when assessed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and immunoblotting. In addition, the plasma form of CR1 exhibited the same structural phenotype as did receptor from erythrocytes of the same individual. CR1 antigen purified from plasma was as active as CR1 from erythrocytes in promoting the cleavage by factor I of C3b to iC3b, C3c, and C3dg. Therefore, a functionally and structurally intact form of **soluble CR1** resides in plasma.

L14 ANSWER 21 OF 26 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1997:369823 BIOSIS
DOCUMENT NUMBER: PREV199799669026
TITLE: The rat is a suitable model to test human decay accelerating factor.
AUTHOR(S): Zaidi, A.; Watkins, N. J.; Harrison, R.; Savill, C. M.; White, D. J. G.
CORPORATE SOURCE: Imutran Ltd., Douglas House, 18 Trumpington Road, Cambridge
UK
SOURCE: Experimental and Clinical Immunogenetics, (1997) Vol. 14, No. 1, pp. 90.
Meeting Info.: 6th European Meeting on Complement in Human Disease Innsbruck, Austria March 12-15, 1997
ISSN: 0254-9670.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L14 ANSWER 22 OF 26 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 199[redacted]69421 BIOSIS
DOCUMENT NUMBER: PREV[redacted]9699091777
TITLE: Complement activation on human neuroblastoma cell lines in vitro: Route of activation and expression of functional complement regulatory proteins.
AUTHOR(S): Gasque, Philippe; Thomas, Anne; Fontaine, Marc; Morgan, B. Paul (1)
CORPORATE SOURCE: (1) Dep. Med. Biochem., Univ. Wales Coll. Med., Heath Park,
Cardiff CF4 4XN UK
SOURCE: Journal of Neuroimmunology, (1996) Vol. 66, No. 1-2, pp. 29-40.
ISSN: 0165-5728.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Two human neuroblastoma cell lines activated the classical pathway of complement in serum. Activation caused the opsonisation of these cells with complement fragments but with moderate cell killing. Neuroblastoma expressed regulators MCP and CD59 but did not express DAF or CR1. Neutralisation of CD59 rendered the cells susceptible to killing. Neuroblastoma also expressed C1-inhibitor, factor H, clusterin and S-protein. Expression of several regulators was enhanced by incubation with cytokines. Complement inhibition using **soluble CR1** markedly reduced opsonisation and killing of neuroblastoma. Our results suggest that complement might play a role in neuronal loss and that treatment with complement inhibitors might be of therapeutic value.

L14 ANSWER 23 OF 26 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:326857 BIOSIS
DOCUMENT NUMBER: PREV199497339857
TITLE: Synergistic inhibition of complement activation by **soluble CR1** and a low M-R protease inhibitor, BRL24894A.
AUTHOR(S): Mossakowska, Danuta E. (1); Scesney, Susanne M.; Marsh, Henry C.; Smith, Richard A. G. (1)
CORPORATE SOURCE: (1) Dep. Biotechnol., SmithKline Beecham, Harlow, Essex CM19 5AD UK
SOURCE: Journal of Cellular Biochemistry Supplement, (1994) Vol. 0,
No. 18D, pp. 158.
Meeting Info.: Keystone Symposium on Structural and Molecular Biology of Protease Function and Inhibition Santa
Fe, New Mexico, USA March 5-12, 1994
ISSN: 0733-1959.
DOCUMENT TYPE: Conference
LANGUAGE: English

L14 ANSWER 24 OF 26 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:148690 BIOSIS
DOCUMENT NUMBER: PREV199497161690
TITLE: Effects of inhibition of complement activation using recombinant **soluble CR1** on release of IL8 and neutrophil activation in a model of acute inflammation.
AUTHOR(S): Finn, Adam; Morgan, B. Paul; Rebuck, Naomi; Rogers, Catherine A.; Moat, Neil
CORPORATE SOURCE: Dep. Paediatr., Univ. Sheffield, Sheffield S10 2TH UK
SOURCE: Journal of Cellular Biochemistry Supplement, (1994) Vol. 0,
No. 188, pp. 317.
Meeting Info.: Keystone Symposium on the Cellular and Molecular Regulation of the Acute Inflammatory Response Tamarron, Colorado, USA February 7-12, 1994
ISSN: 0733-1959.
DOCUMENT TYPE: Conference
LANGUAGE: English

L14 ANSWER 25 OF 26 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 199[redacted]37749 BIOSIS
DOCUMENT NUMBER: BR4[redacted]23449
TITLE: **SOLUBLE CR1** BRL 55730 SCR1 SUPPRESSES GLOMERULAR INJURY IN THREE TYPES OF COMPLEMENT C-DEPENDENT GLOMERULONEPHRITIS GN.
AUTHOR(S): COUSER W; JOHNSON R; PRITZL P; CAMPBELL C; YOUNG B; YEH G; TOTH C A; SHAW D; RUDOLPH A
CORPORATE SOURCE: UNIV. WASH., SEATTLE, WASH.
SOURCE: 25TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF NEPHROLOGY, BALTIMORE, MARYLAND, USA, NOVEMBER 15-18, 1992. J AM SOC NEPHROL, (1992) 3 (3), 581.
CODEN: JASNEU. ISSN: 1046-6673.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L14 ANSWER 26 OF 26 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1990:321570 BIOSIS
DOCUMENT NUMBER: BR39:28906
TITLE: RECOMBINANT **SOLUBLE CR1** SUPPRESSES COMPLEMENT ACTIVATION INFLAMMATION AND NECROSIS ASSOCIATED WITH REPERFUSION OF ISCHEMIC MYOCARDIUM.
AUTHOR(S): WEISMAN H F; BARTOW T; LEPPO M K; BOYLE M P; MARSH H C; CARSON G R; WEISFELDT M L; FEARON D T
CORPORATE SOURCE: JOHNS HOPKINS SCH. MED., BALTIMORE, MD.
SOURCE: MEETING OF THE ASSOCIATION OF AMERICAN PHYSICIANS, THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, AND THE AMERICAN FEDERATION FOR CLINICAL RESEARCH, WASHINGTON, D.C., USA, MAY 4-7, 1990. CLIN RES, (1990) 38 (2), 287A.
CODEN: CLREAS. ISSN: 0009-9279.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

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Peitzsch et al., Biochemistry 32:10436-10443, 1993

McLaughlin et al., TIBS 20:272-276, 1994

Blackshear et al., Biol. Chem. 268(1501-1504), 1993

Thank you,

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Minireview

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The MARCKS Family of Cellular Protein Kinase C Substrates

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Stimulation of membrane polyphosphoinositol turnover is one of the most commonly employed signal transduction systems in normal physiology. The two second messengers generated by this reaction include inositol 1,4,5-trisphosphate, which can transiently elevate intracellular calcium levels, and diacylglycerols, which can activate most isoforms of protein kinase C (PKC).[1] Activation of members of this family of at least 10 isoenzymes leads to a wide variety of physiological effects, including synthesis of macromolecules, activation of transport systems, secretion of hormones, contraction of muscle, and many others.

However, the molecular steps between the activation of PKC and the resultant biological responses are largely unknown. One approach to elucidating these molecular pathways is to study direct cellular substrates for PKC in the hope that the knowledge gained will help to explain downstream events.

This review will focus on one class of PKC substrates of which the prototype is the myristoylated, alanine-rich C kinase substrate or MARCKS protein. This protein, also referred to as 87 kDa, 80K, phosphomyristin, etc. has been employed for at least a decade as an indicator or marker for PKC activation in intact cells; early studies of this type have been reviewed previously (1, 2). This review will concentrate on data that have accrued since the elucidation of the primary sequence of the protein, particularly potential structure:function relationships.

Structure of the MARCKS Protein

The primary sequences of the bovine (3), chicken (4), mouse (5, 6), rat (7), and human (8, 9) proteins have been published (Fig. 1). Recent genetic evidence indicates that these gene products represent species variants of the same protein (10) (see below).

The sizes of the proteins range from 31,891 Da (bovine) to 27,732 (chicken). All migrate with anomalously high M_r on SDS gels, ranging from M_r 87,000 (bovine) to 60,000 (chicken). This is thought to be due to the rodlike shape of the protein, demonstrated by both sedimentation (11, 12) and electron microscopic metal coating techniques (13); this last technique was used to determine the dimensions of the bovine protein, about 4.4 × 36 nm (13). All are very alanine-rich, ranging from 31% (bovine) to 27% (chicken). There are no tyrosines or tryptophans in any of the proteins, and the only methionine is the initiator methionine, which is cleaved in preparation for amino-terminal myristoylation of the protein; this accounts for the general failure to label the protein with [^{35}S]methionine. The proteins are very acidic, with calculated pIs ranging from 4.12 (rat) to 4.42 (bovine). They do not contain significant regions of hydrophobicity, although they are largely membrane-associated in most cells and tissues (see below).

There are three major regions of sequence conservation (Fig. 1). The first consists of the amino terminus, the first 14 amino acids of which are identical in all five species. This conserved domain conforms to the consensus sequence proposed for amino-terminal myristoylation (14); indeed, MARCKS is myristoylated in intact cells (15–18). A second region of sequence conservation surrounds the site of intron splicing in the bovine, human, and mouse (3, 8, 10); differential use of this splice site accounts for the two forms of mRNA (about 2.6 and 4.5 kb) found on Northern analysis.

A final conserved region is a 25-amino acid basic domain that contains the known sites of PKC-catalyzed phosphorylation (19, 20). This phosphorylation site domain (PSD) is extremely basic, with a pI of 12.2 in a protein with an overall acidic pI of less than 4.5. At least three of the four serine residues in this region of the bovine and chicken proteins can be phosphorylated by PKC, both *in vivo* and *in vitro* (see below). The mouse and rat proteins contain a fifth serine in place of the asparagine residue common to the other three species. This basic region of the protein is also involved in calmodulin binding and may play a role in association with actin (see below).

Attributes of the MARCKS Protein

Behavior as a PKC Substrate—MARCKS is a direct substrate for PKC both *in vivo* and *in vitro* (reviewed in Refs. 20 and 21). The bovine and chicken proteins can be phosphorylated *in vitro* to a stoichiometry of about 3 mol/mol (19, 20); however, a recent study (12) achieved approximately 4 mol/mol phosphorylation of the purified, membrane-associated MARCKS protein from bovine brain, compared with 3 mol/mol for the purified cytosolic form. Tryptic digestion of the phosphorylated proteins revealed four distinct phosphorylated serines, all of which are clustered in the 25-amino acid PSD (19). Based on a comparison of tryptic maps and other evidence, these same four serines appear to be phosphorylated *in vivo* (19). In a recent study of PKC-catalyzed phosphorylation of a synthetic PSD peptide, only the first, second, and fourth serines were phosphorylated (22), and the third serine was noted to be in a poor context for PKC phosphorylation. However, a synthetic peptide in which only the third serine remained, with the others replaced by alanine, was still a high affinity substrate for PKC *in vitro* (20). In any case, all phosphorylation sites appear to be clustered in the PSD; when plasmids containing mutant bovine or human MARCKS cDNAs in which all four of these serines had been mutated to cysteines or glycines were transfected into fibroblasts, no phosphorylation of the expressed MARCKS protein could be detected, despite evidence that it was being expressed at levels comparable with those of the endogenous protein.[2]

A synthetic PSD peptide was an excellent substrate for both PKC and its active proteolytic fragment, protein kinase M (PKM) ($S_{0.5}$ = 20 nM; 20). The reaction displayed prominent positive cooperativity. Both high affinity phosphorylation by PKM and positive cooperativity were also observed in a recent study of intact protein phosphorylation.[3]

Neither the PSD peptide nor the intact MARCKS protein was a good substrate for any other protein kinases tested (20), including cAMP- and cGMP-dependent protein kinases and calmodulin-dependent protein kinases I, II, and III. The results with the cAMP-dependent protein kinase are supported by studies in intact cells, in which phosphorylation of MARCKS could not be stimulated by either dibutyryl cAMP or isoproterenol (23, 24). As might be expected, the peptide in which all four serines were replaced by alanines was a potent inhibitor of both PKC and PKM, with inhibitory constants in the 20–50 nM range (20).

Although the MARCKS PSD peptide is a substrate for PKC ε as well as conventional species (25), almost nothing is known about its comparative behavior as a substrate for the 10 or more PKC isoenzymes described to date.

[1] The abbreviations used are: PKC, protein kinase C; MARCKS, myristoylated, alanine-rich C kinase substrate; PKM, protein kinase M; PSD, phosphorylation site domain; MRP, MARCKS-related protein.

[2] D. J. Stumpo and P. J. Blackshear, unpublished data.

[3] G. M. Verghese, J. D. Johnson, and P. J. Blackshear, manuscript in preparation.

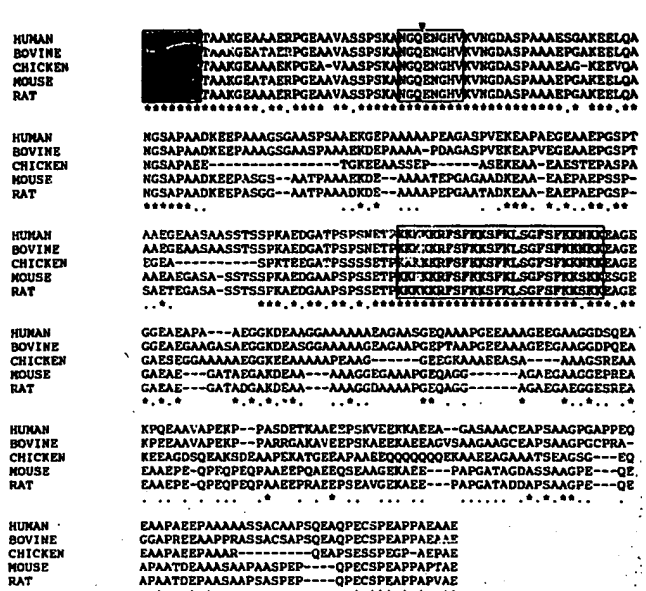

FIG. 1. **Alignment of MARCKS proteins from various animal species.** Sequences were aligned with the program Clustal (60), a generous gift from Dr. D. G. Higgins, European Molecular Biology Laboratory, Heidelberg, Germany. *Asterisks* indicate amino acid identities; *dots* indicate amino acid similarity; *hyphens* indicate gaps inserted to permit optimal alignment. The sources of the sequences used are described (34) except for the human sequence, which was modified as in Ref. 9. The *dark box* indicates the amino-terminal myristoylation site; the *clear box* indicates the region around the site of intron splicing that is similar to a conserved domain in the mannose 6-phosphate/insulin-like growth factor II receptor; the site of intron splicing in the human, bovine, and mouse genes is indicated by the *arrowhead*; and the *lightly shaded box* indicates the phosphorylation site domain (PSD).

Calmodulin Binding—MARCKS was found to bind calmodulin in a Ca^{2+}-dependent manner (26). Furthermore, as in the case of neuromodulin or GAP-43 (27–29), calmodulin binding could be prevented by PKC phosphorylation of MARCKS (26). The calmodulin-binding domain turned out to be identical to the phosphorylation site domain (26). Both the PSD peptide and the intact protein bind calmodulin with high affinity (K_d 2–5 nM), with half-maximal effective calcium concentrations being about 400 nM (30). Phosphorylation of the peptide decreases its affinity for calmodulin by about 200-fold, and rapidly disrupts pre-existing peptide-calmodulin complexes (30). Although the PSD peptide fits one criterion for a calmodulin-binding domain (31) in that it can be placed in the configuration of an amphiphilic α-helix, with basic residues along one side and hydrophobic residues along the other (26), recent circular dichroism data suggest that both the peptide (32) and the intact protein (12) exist in solution as a random coil. The α-helix requirement is further negated by recent data concerning the analogous peptide from the F52, MRP or MacMARCKS protein, which contains a proline in the middle of the calmodulin-binding domain (33–35) (see below).

These findings raised the possibility that the MARCKS proteins could serve as a reservoir for calmodulin in cells in states of PKC inactivity, perhaps releasing calmodulin from membranes upon PKC activation. A similar role has been proposed for neuromodulin (27–29). The relative abundances of MARCKS and calmodulin are consistent with this possibility; in brain, the concentration of calmodulin has been estimated to be about 60 μM (36), whereas that of MARCKS is about 12 μM (11).

Further support for this idea comes from two lines of recent evidence. Studies in cultured neuronal cells demonstrated that activation of PKC leads to increases in cytosolic immunoreactive calmodulin, with commensurate decreases in membrane-associated calmodulin (37, 37a); this result is compatible with MARCKS and other similar proteins "tethering" calmodulin to cellular membranes, perhaps through their fatty acyl groups, until calmodulin is released by PKC-catalyzed phosphorylation of the tethering molecule. In addition, recent microinjection studies in *Paramecium* suggest that the MARCKS PSD peptide forms a complex with calmodulin in the intact cells at prevailing calcium concentrations and that this complex is disrupted by phorbol ester activation of PKC (38). Direct proof of MARCKS-calmodulin complex formation and disruption by PKC is, however, lacking in intact vertebrate cells.

Myristoylation—MARCKS was first found to be myristoylated by Aderem *et al.* (15) in mouse macrophages. Initial suggestions that the protein might be myristoylated post-translationally in an agonist-dependent manner were not supported by James and Olson (17), who demonstrated in other cells that the protein was myristoylated co-translationally. Another recent study (39) showed, however, that there exists a pool of non-myristoylated MARCKS in rat brain, which conceivably could be myristoylated post-translationally. The question of possible post-translational myristoylation and demyristoylation, perhaps stimulated by agonists, has therefore not been completely resolved.

When normal and mutant chicken MARCKS proteins were expressed in mammalian cells (16), an amino-terminal replacement of glycine by alanine completely prevented both myristoylation and membrane association. The mutant protein was, however, still an excellent substrate for PKC in intact cells. Therefore, in contrast to $pp60^{v\text{-}src}$ (40–42), MARCKS was a substrate for PKC *in vivo* whether it was myristoylated and membrane-associated or not. A possible corollary is that at least some species of PKC can phosphorylate both membrane-associated and cytosolic proteins in cells.

An interesting question is whether the MARCKS protein associates with plasma membranes by binding to a myristate-dependent, cytoplasmic-face protein "receptor," as has recently been demonstrated by Resh and colleagues (43, 44) for $pp60^{v\text{-}src}$. This question was addressed for MARCKS by analyzing the association of *in vitro* translated MARCKS with a cellular membrane preparation (45). Binding to membranes under these conditions was clearly myristate-dependent. However, the myristoylated protein bound normally to membranes after they were either boiled or subjected to extensive trypsin digestion. These data suggested that the binding was not to a cytoplasmic-face protein receptor but probably resulted from hydrophobic interactions between the myristate group and the lipid bilayer (45).

Actin Binding—In murine macrophages, immunoreactive MARCKS was found in a punctate pattern at the interface of the substratum with pseudopodia and filopodia, where it colocalized with vinculin and talin (46). Activation of PKC in these cells led to disappearance of MARCKS from its punctate localization, as well as its release from the plasma membrane; these changes were associated with a major cytoskeletal rearrangement (46).

More recently, Hartwig *et al.* (13) demonstrated that dephosphorylated MARCKS could bind to and cross-link filamentous (but not globular) actin *in vitro*. Cross-linking occurred at low MARCKS:actin ratios, as determined by negative staining with electron microscopy and dynamic light scattering. The dephosphorylated protein decorated the actin filaments with a periodicity of about 20 nm; the phosphorylated protein could bind actin, but less tightly, and could not cross-link actin. Calcium/calmodulin also prevented actin binding and cross-linking by dephosphorylated MARCKS. The involvement of the PSD/calmodulin-binding domain in the actin interaction was confirmed by studies of a 19-amino acid synthetic peptide representing the PSD, which caused actin to aggregate into loose bundles, a phenomenon that was reversed by either PKC-dependent phosphorylation or calcium/calmodulin (13).

On the basis of these data, Hartwig *et al.* (13) speculated that activation of PKC and increases in cytosolic calcium concentrations could regulate the interaction of MARCKS with both the actin cytoskeleton and the plasma membrane of these murine macrophages. In this way, both phosphorylation and calmodulin binding could modulate the ability of MARCKS to serve as a cross-bridge between the substrate-adherent plasma membrane and membrane cytoskeletal actin during chemotaxis (13).

The MARCKS Gene and Its Regulation

MARCKS genomic clones have been isolated and sequenced from human (8) and mouse (10). The human gene (designated *MACS* (8)) has been localized to chromosome 6q21 (8, 10). The mouse gene (designated *Macs* (10)) was localized to chromosome 10, and it was in the middle of a gene linkage group that corresponds to a similar series of genes in the vicinity of human

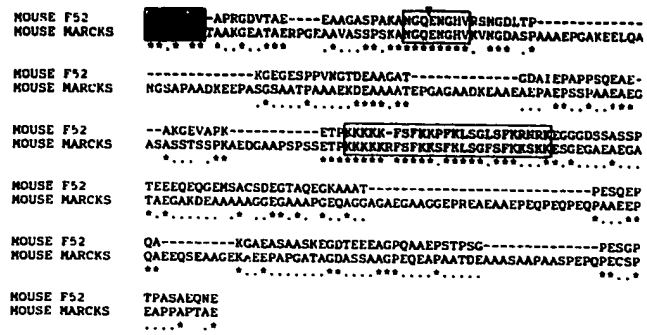

FIG. 2. **Alignment of mouse MARCKS with mouse F52/MacMARCKS/MRP.** This alignment was performed using the program Clustal; symbols and other abbreviations are as described in the legend to Fig. 1. The sources of the sequences used are described in Ref. 34. The *arrowhead* indicates the site of intron splicing in both the mouse MARCKS (10) and F52/MacMARCKS/MRP proteins.[4]

chromosome 6q21 (10). This finding identifies *Macs* as the mouse counterpart of *MACS* rather than as a related mouse gene. An additional piece of evidence supporting this conclusion is the very high degree of sequence identity between the promoter regions of the two genes. The two promoters were 89% identical over the first 612 base pairs and contained at least 59 potential transcription factor binding sites in analogous locations (10).

Both promoters lack TATA boxes, a rather unusual feature for a gene whose expression appears to be highly regulated. The MARCKS gene most closely resembles what Smale and Baltimore (47) have called a Class 1 TATA-less promoter, which have been found primarily in housekeeping genes; however, the multiple modes of regulation of MARCKS gene expression are not typical for a housekeeping gene. These include: 1) tissue-specific expression (3, 48, 49); 2) developmentally regulated expression (50); 3) acute increases in apparent transcription in response to cytokines such as tumor necrosis factor α and lipopolysaccharide (8, 51); 4) changes in mRNA levels (usually decreases) during malignant transformation (52–54); and others. Although numerous potential regulatory elements have been identified in both the mouse and human promoters (10), and at least 110 base pairs appear to be required for expression of the human promoter (8), little is known about the elements in the promoter or elsewhere in the gene that regulate its transcription.

A very interesting form of regulation of MARCKS mRNA and protein levels was identified by Rozengurt and colleagues (6, 55). Similar observations were made by Lindner *et al.* (56), although the time courses of the responses differed markedly in the two studies. Brooks *et al.* (6) noted that MARCKS protein and mRNA levels were profoundly decreased several hours after treatment of Swiss 3T3 cells or mouse embryo fibroblasts with active phorbol esters. Similar effects were observed with physiological activators of PKC, such as bombesin or platelet-derived growth factor (55); they were also seen in cells treated with activators of the cAMP-dependent protein kinase and in platelet-derived growth factor-treated cells after down-regulation of PKC with prolonged phorbol ester treatment. These changes in mRNA levels, which reached a maximal decrease of about 95% after 8 h of bombesin treatment, were not accompanied by changes in MARCKS gene transcription. Thus, activation of PKC, as well as of certain other pathways, appeared to produce profound changes in MARCKS mRNA and protein levels through a post-transcriptional mechanism, perhaps through alterations in mRNA stability.

The F52/MacMARCKS/MRP Protein

A MARCKS relative was recently cloned by screening a mouse cerebellar library for transcripts preferentially expressed in the cerebellum (33, 57). This cDNA clone was labeled F52 (33); its predicted protein structure bore a striking similarity to MARCKS, particularly in the three evolutionarily conserved domains described above (Fig. 2). We expressed this cDNA in *E. coli* and studied the properties of the expressed protein and its related peptides, keeping the F52 appellation (34). In independent work, Li and Aderem (35) cloned the rabbit and mouse versions of this protein and labeled it "MacMARCKS" because f its high level expression in macrophages. We have recently cloned and sequenced the mouse gene encoding this mRNA and mapped its chromosomal location.[4] The gene was designated *Mrp* (for MARCKS-related protein) in mice and *MRP* in humans by the Committee on Standardized Genetic Nomenclature for Mice and the Human Gene Nomenclature Committee, respectively. Because the gene is widely expressed in mouse tissues,[4] and to avoid persistent nomenclature confusion such as that with neurom - dulin/GAP-43/B52, we propose that the protein be called MRP.

The properties of MRP are remarkably similar to those f MARCKS (34, 35). It is considerably smaller (M_r 20,165 in the mouse), migrates very anomalously on SDS-polyacrylamide gels, and is also heat stable, acidic, and alanine-rich, with no tyrosines or tryptophans. It is myristoylated in intact cells and serves as a substrate for *N*-myristoyltransferase in *E. coli* after co-expression (34). It is also an excellent substrate for a mixture of rat brain PKC isozymes, with affinities for the PSD peptide and intact protein[3] of approximately 173 nM (34). Its affinity for calmodulin is approximately the same as that of MARCKS, about 2–5 nM (34).[3] As in the case of MARCKS, phosphorylation by PKC prevents and disrupts calmodulin complex formation with both the PSD peptide and the intact protein. This has been shown *in vitro* (34) and in experiments involving microinjection of MRP peptides into *Paramecium* as described above for MARCKS (38).

Mrp is similar to *Macs* in that it is a relatively simple gene with a single intron, located at the identical site in the mRNA as the intron in *Macs* (Fig. 2). The surrounding protein domain comprises a region of sequence identity among MARCKS proteins from all species studied to date, as well as MRP from mouse, rabbit, and human. This domain contains seven amino acids that are identical to a small motif in the cytoplasmic region of the mannose 6-phosphate/insulin-like growth factor II receptor, again highly conserved among species (34). The function of this small motif is not known in any of these proteins. In the case of the receptor, this is not the domain thought to be responsible for either receptor internalization (58) or signaling (59).

Mrp and *Macs* are located on different chromosomes, and the corresponding human genes are also located on different chromosomes.[4] Unlike *Macs*, which seems to have no related sequences or pseudogenes (although see Ref. 9), mouse *Mrp* appears to be related to more than one DNA sequence, based on Southern blotting. Five of these genomic clones have been mapped and partially characterized.[4] In all cases, the evidence is strong but not conclusive that the related sequences represent pseudogenes. For this reason, the Nomenclature Committee has labeled them *Mrp-rs1* through *Mrp-rs5* (for *Mrp*-related sequences).[4]

Mrp appears to be regulated in many ways analogously to *Macs*. For example, it is strikingly induced in macrophages by lipopolysaccharide (35). In addition, its tissue-specific expression

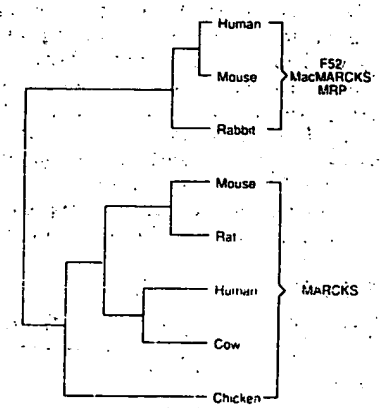

FIG. 3. **Dendrogram of MARCKS family sequences.** This dendrogram was constructed using the program PILEUP, from the Genetics Computer Group, Inc., Madison, WI. The length of the *horizontal lines* is proportional to the difference between two sequences; the *vertical lines* have no significance. This dendrogram does not necessarily represent evolutionary relationships. The sequences used in this alignment are described in the legend to Fig. 1, except for the rabbit F52/MacMARCKS/MRP sequence (35) and the human F52/MacMARCKS/MRP sequence.[3]

[4] D. F. Lobach, M. F. Seldin, and P. J. Blackshear, manuscript in preparation.

is similar to that of *Macs*, although it appears to be much more highly expressed in reproductive tissues than *Macs*.[4] It was surprising, therefore, that the sequences of the two mouse promoters have only limited identity.[4] Promoter elements in both genes that regulate tissue-specific, developmentally regulated, and cytokine-stimulated expression are still to be determined.

It is clear from the similarities between the MARCKS and MRP proteins shown in Fig. 2 that these proteins are members of what now appears to be a small gene family of heat-stable, myristoylated, alanine-rich, calmodulin-binding, probably actin-binding PKC substrates (Fig. 3). Many questions about this family remain to be answered, as enumerated in the preceding paragraphs. In my view, the greatest remaining challenge is the elucidation of the role of these proteins in the intact cell, as potential mediators between activated PKC and the myriad resulting cellular responses.

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